Progress in GM cotton development in public sector

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Content

- Introduction
- Global status
- New GM cotton development
- Public sector cotton biotech in Pakistan
- Issues in GM crop release
- Way forward

Efficiency

USA

Year	Area	Production
1926	42m Acres	18 M.bales
2006	12 m Acres	22 M.Bales

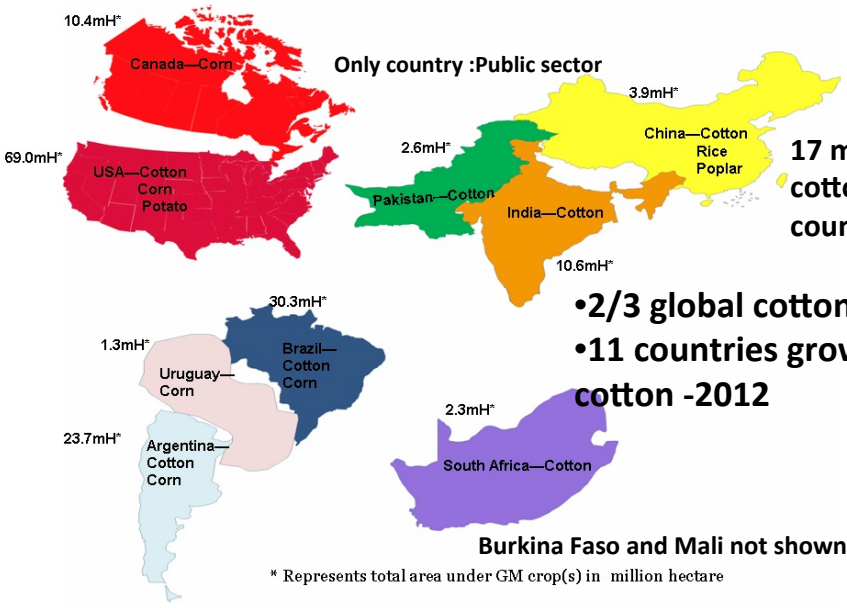
The ability to produce more on less land

USDA Land data utilization record

India

2006	20 m Acres	18 M bales
2011	24.75 m Acres	32 M Bales (bt cotton)

Fig.1 Map showing the distribution of Bt-crops growing on >1.0 million ha in different countries



GM Cotton by Monsanto

Traits	Event/Action	Trade marks
Insect resistance	Cry 1 Ac- Mon 531	Bollgard I- 1996
	Cry 2 Ab- 15985 Lygus control	Bollgard II- 2000 Phase III, Genuity/Bollgard III
Herbicide tolerance	EPSPS-Round up ready Dicamba, Gulfosinate	RR- 1996 SmartStax
Nematode Tolerance	Reinforce , Root knot	Phase 2, 3
Drought tolerance	5-14 genes	Phase III

Du-Pont, Dow,Bayer Crop Sci , Biocentury, Silver land, Hebei etc

Progress in GM cotton development in public sector

- Thea Wilkins, UC Davis now A&M Texas, USA

Fiber ESTs, Several gene constructs for fiber improvement

- Keerti S. Rathore, A&M Texas,USA

Cotton oil quality improvements with seed specific reduction in Gossypol

US Patent 2011/0314572 A1

- CAAS, Beijing, China

Cry 1Ac and CPTI

- CSIRO, Australia
- Nematode/bacterial blight resistance , white fly control

The draft genome of a diploid cotton *Gossypium raimondii*

Nature Genetics 44, 1098–1103 (2012)
doi:10.1038/ng.2371
Nature Genetics | Article Open

日本語要約

Kunbo Wang *et.al*

Published online 26 August 2012

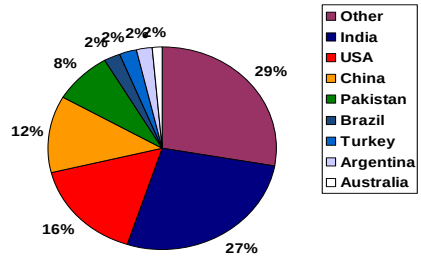
Parameters of different *G. raimondii* genome assemblies

	Draft	*Reference*
Scaffold number	4715	1084
N50 (Megabases)	2.3	18.8
Longest	12.8	52.1
Anchored and oriented % genome	52.4%	98.3%
Gene number	40,976	37,505

Reference sequence by Andrew Patersen, Univ of Georgia, USA

Genome of diploid AA and tetrapoloid will soon be available

Cotton and Pakistan



- **Pakistan Contributes**
  - **Global Area: 8 %**
  - **Production: 12 %**
  - **4th largest producer**

Total Exports	66%
Manufacturing	46%
Textile Exports (2004-05)	$9.505 Bln.
Employment	38%
GDP	8.5%
Investment in Textile (1999-2005)	$ 5.5 Bln.

410 Textile 1200 Ginning mills

- **Threats**
  Abiotic and Biotic stress

What are the requirements?

Political will

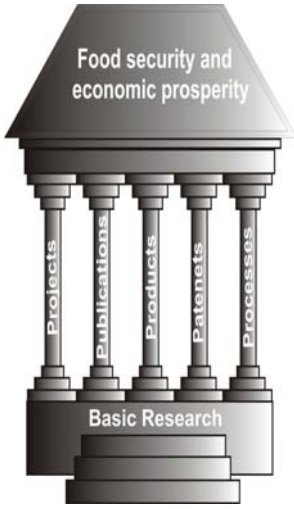
- Infra structure (human and physical)
- Consensus on use of Agri.Biotech,
- Enabling environment

Regulatory frame work

Biosafety laws, IPR, PBR

Acceptance by farmers

- Enormous success of Bt cotton
- Affordable
- Transparent



Pakistan: Present status

8th largest GM area in the world

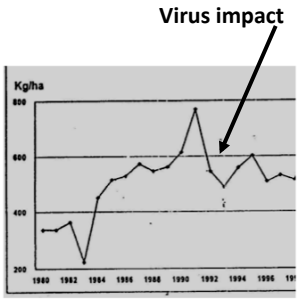
Bt cotton is the only crop
(ISAAA Brief-43,2012)

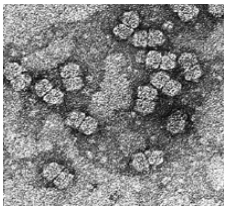
- ***Other GM crops with different traits are at evaluation and approval stage***

Cotton leaf curl virus epidemic in Pakistan

Epidemic level in 1992

Heavy losses to Pakistan cotton
(5 billion US dollars)





Cotton Biotech in Pakistan

•**NIBGE (1994 - onward):** ***a national asset***

Assembly of dynamic team (Together Everybody Achieve More)

✓ Establishment of infrastructure

✓ Acquiring of technology: Multipronged strategy

- Foreign collaboration:

JIC, SCRI, ILTAB, A&M Texa, Univ. of Arizona, Univ. of Georgia, CSIRO,

- Reverse Engineering: Cry1Ac

(1998- no patent law, no biosafety issues)

- Acquiring new genes: ICGEB

(MTA - Cry1Ac, Vip, Cry5A, etc)

- Making new genes:

Hvt, Cry1Ac, Cry2Ab, Cry5A, Cambia vector

Cotton Biotechnology Programme

Engineering for:

- **Virus (CLCuV) resistance**
- **Insect resistance**
- **Drought / salinity / heat tolerance**
- **Herbicide tolerance**
- **Fiber improvement**

Tissue Culture of Cotton

- Recalcitrant
- Tissue culture responsive

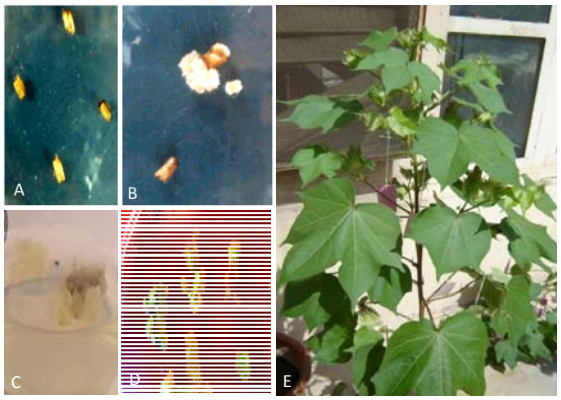
  -Coker- Acala
  -Local varieties

Low yielding

Heat intolerant

Highly susceptible to CLCuV

Undesirable fiber characteristics



Different steps of tissue culture in cotton. A) hypocotyl section excised from germinated seeds placed on callusing media; b) callus induced on hypocotyl sections; c) proliferation of embryogenic callus; d) formation of embryoids in-vitro; e) a tissue culture regenerated plant

Courtesy Dr. Shaheen Aftab

2. Non Conventional resistance

A. Pathogen Derived Resistance (virus/vector)

i. Protein mediated resistance
ii. RNAi/miRNA based resistance
iii. Defective or DNA interference

i. Protein mediated resistance against geminiviruses

Full length or truncated genes have been used

Rep (TbMV, ACMV, ToLCuNDV, TYLCV, CLCuD, ToMoV, BGMV, MYMV)
MP (TGMV, ToMoV, BGMV)
CP (TYLCV)

Constructs developed at NIBGE for virus resistance

A. Pathogen Derived Resistance (against vector)

1. Artificial miRNA based construct for whitefly resistance (Construct 1)

A. Pathogen Derived Resistance (against geminivirus)

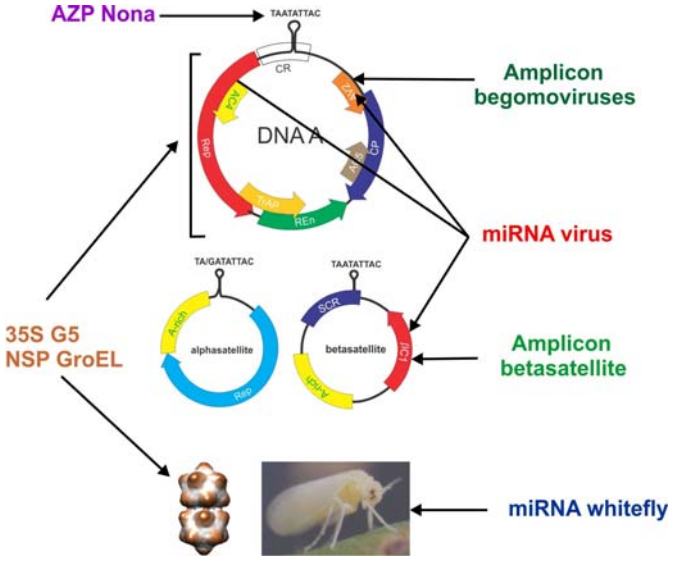
2. Amplicon based RNAi construct targeting begomoviruses (Construct 2)
3. Artificial miRNA based construct for virus resistance (Construct 3)
4. Amplicon based RNAi construct targeting betasatellite (Construct 4)

Construct handed over to CEMB, July 5, 2012

B. Non pathogen derived resistance (protein mediated against viruses)

6. 2X35S-AZP Nona (Construct 6)
5. NSP- GroEL and 2X35S-G5 (Construct 5)

Constructs and their targets



Novel sources of broad-spectrum virus resistance

Technology	Status
✓RNAi based silencing of V2	Cotton
✓Micro-RNA based resistance	Tobacco
✓Inducible DI molecules	Being constructed
✓Single-stranded DNA binding proteins	Tobacco transformation
✓Zinc finger resistance	Being constructed
✓Single-stranded DNA binding proteins	Tobacco transformation
✓GroEL based resistance	Tobacco transformation

Long-term solution

Pyramiding of natural and engineered resistance sources

Scientists are Congratulated for Developing Miracle CLCV Resistant Cotton Plant

PARB NEWSLETTER –october,2012, Vol III

Punjab Agriculture Minister Malik Ahmad Ali Aulakh and Chief Executive PARB Dr. Mubarik Ali visited Center of Excellence in Molecular Bi-ology (CEMB) in Punjab University and congratulated the scientists for **developing cotton GMO which can effectively resist the CLCV growth in the plant.**

Cotton Leaf Curl Virus (CLCV) is devastating cot-ton production and woes the cotton farmers every year. The losses due to the disease are estimated 3 million bales worth of approximately Rs. 250-300 billion depending upon the cotton prices.

PARB started a project in collaboration with CEMB and Institute of Agricultural Sciences (IAS) in Punjab University in 2009 to develop GMO that can effectively resist the virus growth in cotton plant with a cost of Rs. 29 million. The GMO construct against the virus was built in Toronto University. It was incorporated in the best cotton varieties of Punjab in collaboration with IAS and CEMB. The methodology used to build the construct is called **RNAi technique**.

The scientists told the minister that international and national collaboration developed through PARB played a critical role in the success of this project. They informed the minister that couple of confirma-tory test is needed before the seed of the GMO can be given to the farmers. These research stages will be completed within 1-2 years.

The minister for agriculture gratitude Dr. Mubarik Ali for initiating this project, his planning and coordination efforts that brought the success. He emphasized to speed up the work so that the fruit of the scientific success can reach to the farmers as soon as possible. He assured all support to complete the project as soon as possible.

MOLECULAR VIROLOGY IN PAKISTAN

- **Largest group in NIBGE/ Pakistan**
- **Earned national/ international fame "Mecca of Geminivirus Research"**
- **Prof. Dr. Rob Briddon, JIC, UK, staying at NIBGE since 2003**
- **Diversity of Cotton Leaf Curl Virus**
- **Transmission of virus (grafting, whitefly, infectious clone)**
- **Diagnostic Tools for identification**
- **Correct Identification of alternate hosts**
- **Providing gene/ vector for PDR Genetic engineering**
- **Expanded to other crops**

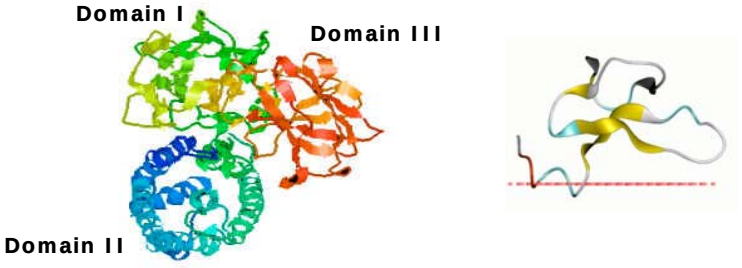
Genes for Insect Resistance Developed and used at NIBGE

First Generation of Transgenic Plants

Cry1Ac	Characterized in tobacco and cotton	Highly effective against *Helicoverpa*
Vip3A	Characterized in tobacco	Highly effective against *Helicoverpa, ..?*
Cry2Ab	Gene constructs developed Transformation of tobacco and cotton in progress	Highly effective against *Spodoptera*
Hvt	Characterized in tobacco and cotton	Highly effective against *Helicoverpa, Spodoptera,* sap-sucking insects?
Lectins	Characterized in tobacco	Effective against mealybugs

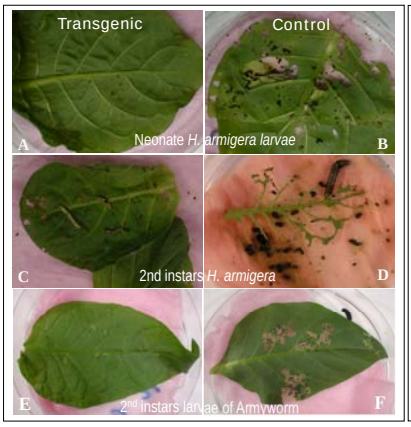
Funnel Web Spider
Master insecticide chemist

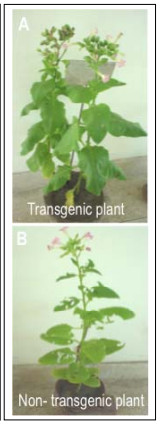
- **Australian funnel web spider - *Hadronyche versuta* one of the most deadliest spiders**
- **Its venom has a complex cocktail of insecticidal proteins**
- **Venom contain >100 peptide toxins**
- **Most of them are expected to have insecticidal effect**
- **One of these toxins ω-atracotoxin-Hv1 characterized biochemically**
- **insecticidal activity against the larvae of butterflies and moths**

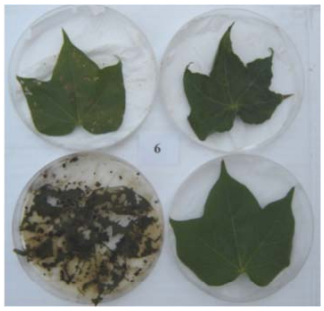


Structural comparison of Bt cry protein and the spider protein (Versutatoxin)

Insect Bioassays - Hvt plants







2nd instar larvae of *Helicoverpa armigera*

◄ 2nd instars larvae of Armyworm.

Mukhtar et al. (2004) Patent No. 139032

Khan et al. (2006) Transgenic Research 15: 349-357

Second Generation of Transgenic Plants

Cry1Ac + Hvt	Characterized in tobacco being transformed in cotton	Transgenic tobacco are highly effective against *Helicoverpa and Spodoptera*
Vip3A + Hvt	Characterized in tobacco, being transformed in cotton	Highly effective against *Helicoverpa and Spodoptera*
Cry1Ac + Cry2Ab	Characterized in tobacco Transformation of cotton in progress	Controls both *Helicoverpa* and *Spodoptera*
Hvt + lectin	Not yet initiated	--

Abiotic stress tolerance

- Heat
- Drought
- Salt
- Cold

Genes

- AVP1
- AtNHX1
- HSR1

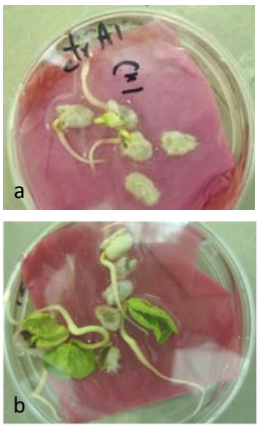
Genes available for Abiotic stress at NIBGE

1. ***AVP1, AVP1-D*** **(2 clones)(Gain of function mutant): pyrophosphatase energy generating proton pump from *Arabidopsis* for drought and salt tolerance**
2. ***AtNHX1*** **(2 clones -cDNA and genomic clone): Na+/H+ antiporter involved in salt and drought stress**
3. ***DREB1A*** **(2 clones -under rd29 and FMV promoters): transcription factor that activate stress genes. cDNA cloned from A. thaliana for drought**
4. ***HVA1*** **(2 clones - 2-under rd29 and FMV promoters): Late Embryogenesis Abundance (LEA) protein. cDNA cloned from Barley for drought**
5. ***WXP1*** **(2 clones - 2-under rd29 and FMV promoters): Wax gene from M. truncatula . cDNA obtained from Noble Foundation for drought**
6. ***AtNCED3*** **(2 clones - under rd29 and FMV promoters): Arabidopsis gene- ABA pathway gene that is involved in closure of stomata for drought**
7. ***HKT1*** **(3 clones) - isolated from barley and cloned under three promoetsr Act1, Uni and 35S for salt tolerance**
8. ***LVP1*** **(3 clones) - AVP1 homologue isolated from Kallar grass and cloned under three promoters Act1, Ubi and 35S for salt and drought tolerance**
9. **Alanine Aminotransferase (2 clones of AlaAt)- synthetic gene for enhancing nitrogen use efficiency**

Total genes/clones: 21

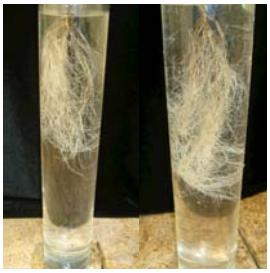
28

AVP1 transgenic cotton for abiotic stress tolerance and phosphorus use efficiency



Seed germination in the control (a) and transgenic (b) plants

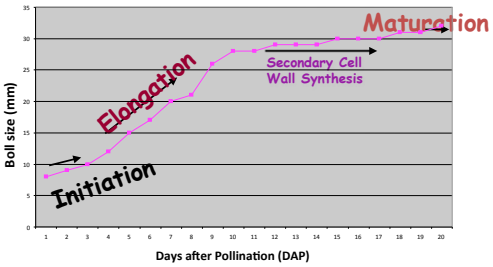
Effect of AVP1 on growth of plants in the control (left) and transgenic (right) plants



Root growth of un-transformed (control) and transgenic plants)

Courtesy; Dr. Shaheen Aftab

Cotton Fiber Development Stages



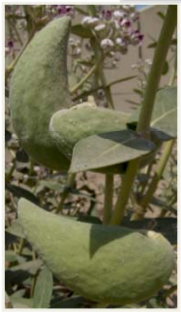
Cotton

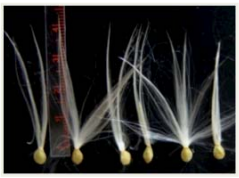
Family: ***Malvaceae*** **Genus:** ***Gossypium*** **Species:** ***hirsutum*** **2n=4x=52**

Aftab Bashir, ABD, NIBGE

Gene Resources

1) Cotton fibers
2) *Calotropis procera* fibers





Aftab Bashir, ABD, NIBGE

Development of gene resources for cotton improvement

Accomplished:

i) Six Cotton Fiber cDNA Libraries
ii) One *Calotropis procera* seed fiber cDNA library
iii) One *G. arboreum* leaf cDNA library

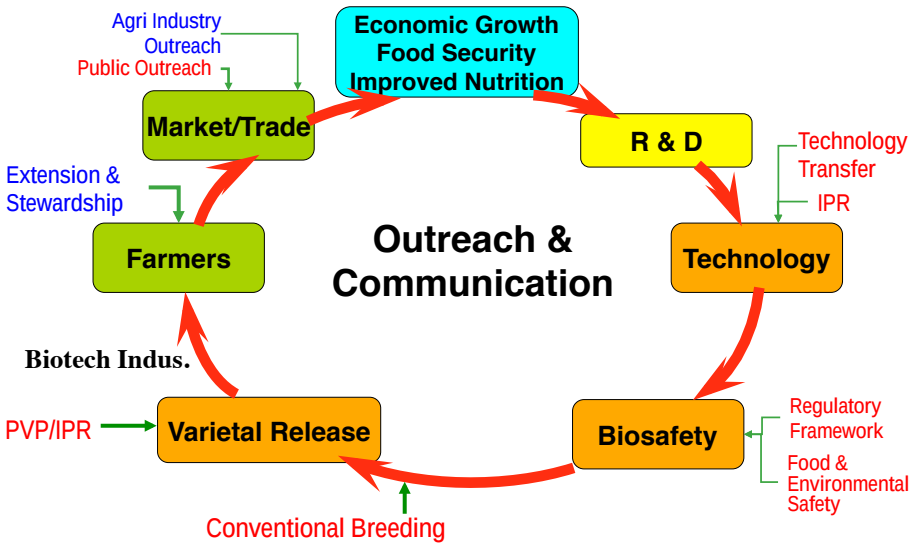
About 1500 ESTs established from the above libraries

Isolated genes being used for cotton fiber improvement

i) Expansins (cotton and *C. procera*)
ii) Sucrose synthase (cotton)

Genes for potential useful traits isolated and being characterized

Comprehensive Approach to Agricultural Biotechnology

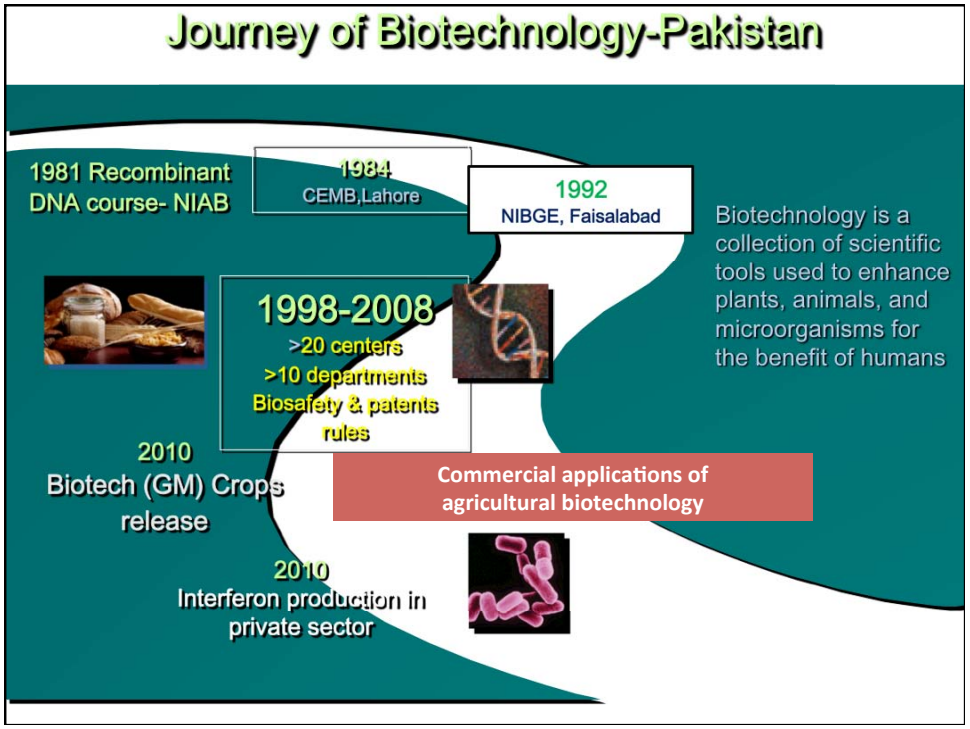


APPROVAL OF GM CASES WITH NBC/ PAK-EPA

- **IBC** **36 (notified)** **Public Sector + Private (National – MNCs)**
- **TAC** **16 Meetings Held**
- **NBC** **09 Meetings (last 15 February, 2011)**
- **Status**
- **Total Cases Submitted** **155**
- **Cases notified** **118**

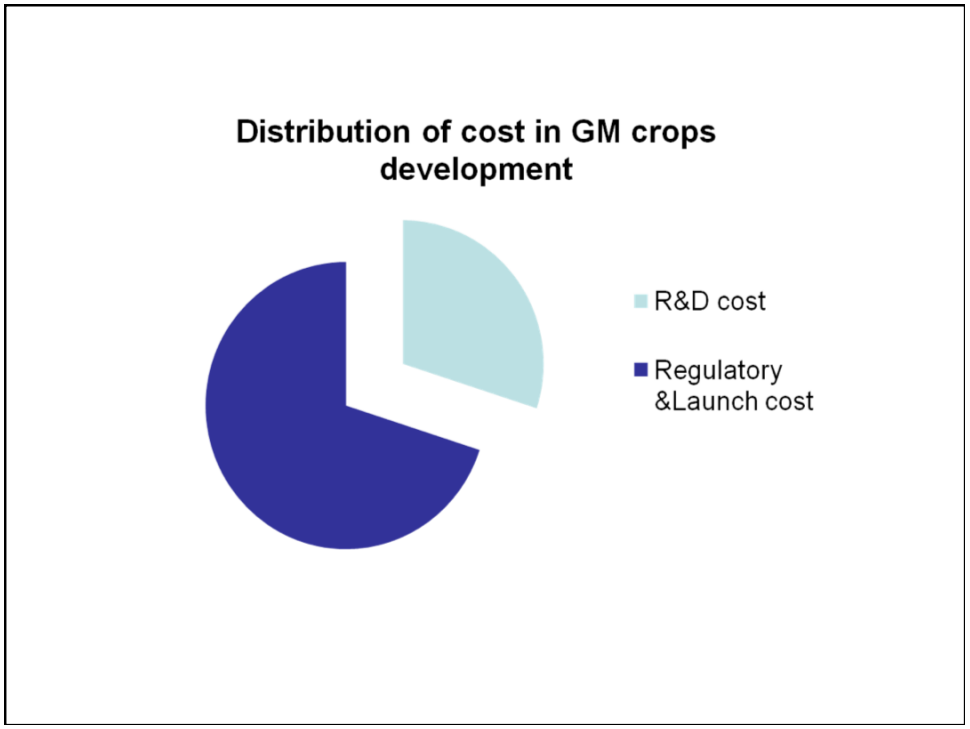
- **Labs + GH + Field Studies** **109**
- **Commercial Approval**
- **(De-regulated/ Exempt Status)** **09 (Bt – Cotton)**
- (*Pak Biosafety Rules – 2005)*

*Updated on 28th March, 2012



GM Crop Release: Complex Issues

- **R & D:**
  Capacity, infrastructure, lack of co-ordination
- **Biosafety:**
  Lack of trained human resource, infrastructure, finances
- **PBR/ Patents:**
  Liberal patent policy, modifications of PBRs
- **Monitoring/ Evaluation:**
  IPM, toxin level, purity, infringments,litigations



Biosafety and the Patents- *Major Impediments*

Path to success

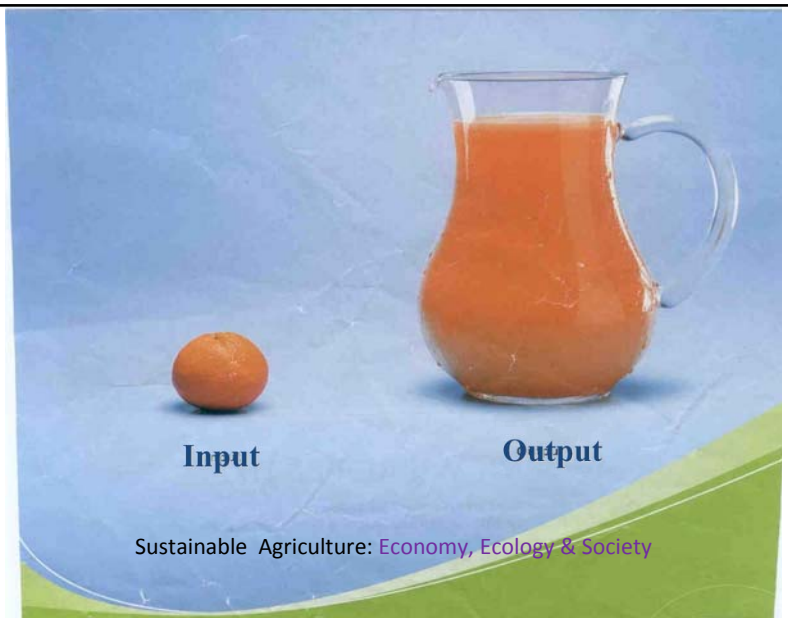
- One trait-one crop-one team
- Preferably adopt success stories
- Stringent filters at each stage/strict time scale
- Ruthless screening
- Close linkages with breeders
- Development of GO-NGO partnership

Conclusions-Way Forward

- Existing GM crops benefit producers, farmers and consumers in both industrialized and developing countries
- Regulatory and IP costs must be kept realistic to ensure safety while allowing GM crops to benefit the rural and urban poor, especially in developing countries
- ***Strengthen the public R&D system***

WAY FORWARD--contd

- **Leave safe haven of research-use-only to applications of widespread public use.**
- **A 'tsunami" of genomic data and information is coming**
- **GM technology and modern breeding is going to stay and expand**
- **Assemble a multidisciplinary team and focus on one crop and few tested traits.**
- **Development of workable business model**
- **Level-playing field and win-win situation**



GET MORE OUT OF SMART AND EFFICIENT GM CROPS: the most adopted technology of 21st century, Times June 2010

PAEC