GMO Status and GMO Analysis in India

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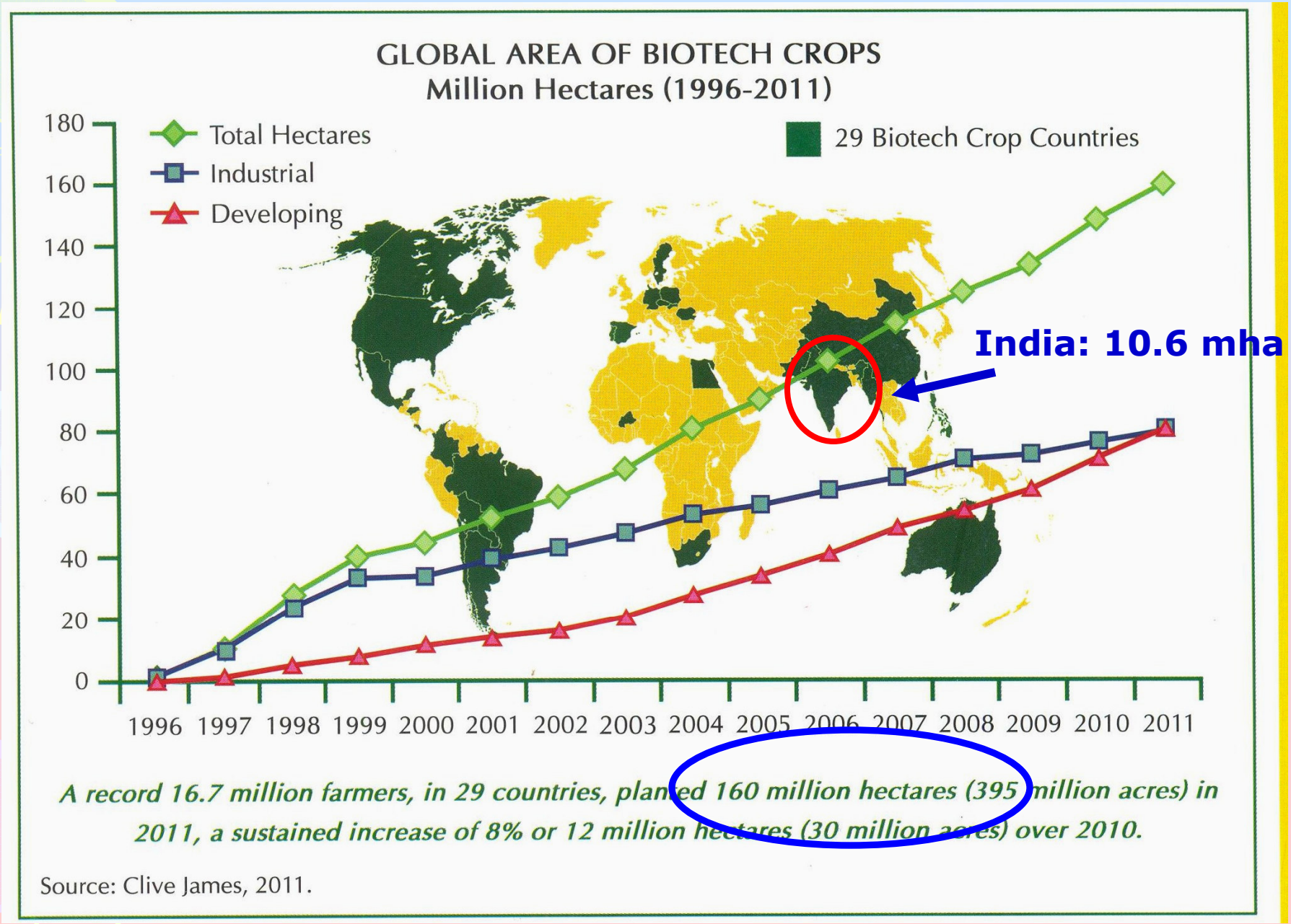

GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996-2011)
Total Hectares
Industrial
Developing
29 Biotech Crop Countries
180
160
140
120
100
80
60
40
20
0
1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011
India: 10.6 mha
A record 16.7 million farmers, in 29 countries, planted 160 million hectares (395 million acres) in 2011, a sustained increase of 8% or 12 million hectares (30 million acres) over 2010.
Source: Clive James, 2011.

National Regulatory Mechanism for GM Crops

National Regulatory Mechanism

Ministry of Environment and Forests

- » *Environment Protection Act (EPA)*
- » *Genetic Engineering Appraisal Committee (GEAC)*

Ministry of Science and Technology

- Department of Biotechnology (DBT)
  - » Review Committee on Genetic Manipulation (RCGM)

Ministry of Agriculture and Cooperation

- Department of Agricultural Research and Education (DARE) / Indian Council of Agricultural Research (ICAR)
  - » National Bureau of Plant Genetic Resources (NBPGR)
- Department of Agriculture and Cooperation
  - » *Plant Quarantine (Regulation of Import into India) Order 2003*

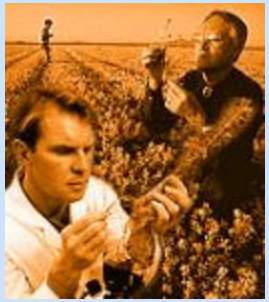
GM Crops are governed by

- **Environment Protection Act, 1986**
  - **Came into force from 23.05.1986**
- **Rules, 1989 on GMOs**
  - **Notified on 05.12.1989**
  - **Came into force from 01.10.1993**

GM Crops are also governed by

- Industries (Development & Regulation) Act, 1951
  - New Industrial Policy & Procedures, 1991
- Seeds Act, 1966
- Seeds Rules, 1968
- Seeds (Control) Order, 1983
- Seeds Policy, 1988, 2002
- Protection of Plant Varieties and Farmers' Rights Act, 2001

Biosafety regulations



Applicant/investigator

* To note
* To approve
* Recommend & seek approval of RCGM

IBSC

MEC

* Visit trial sites
* Analyse data
* Inspect facilities
* Recommend safe

* Recommend to generate biosafety & agronomic data

RCGM

ICAR

* Generate agronomic data
* Recommend for release

* Approve large scale use
* Approve open release in en
* Inform decision to all concern

GEAC

Applicant/investigator

State Govts permission

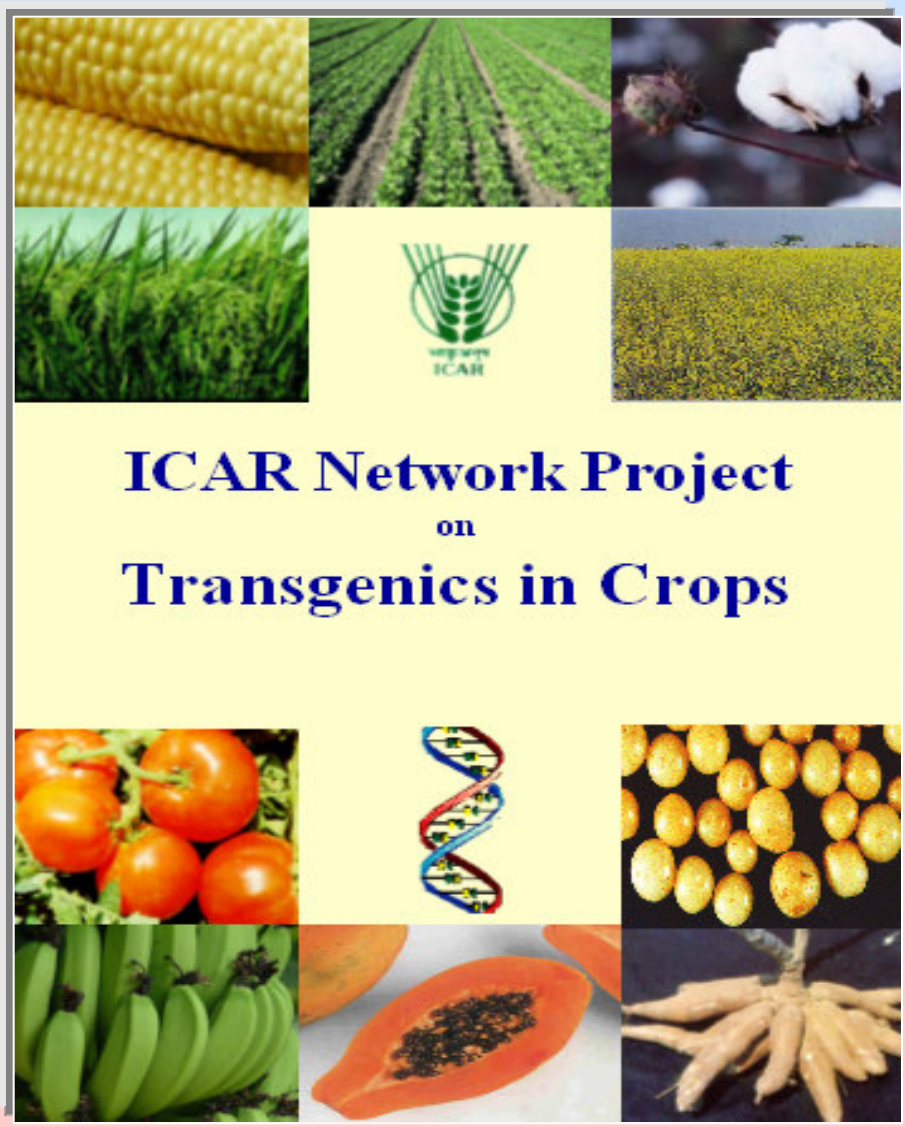
Release for commercial Agriculture

Status of GM Crops in India

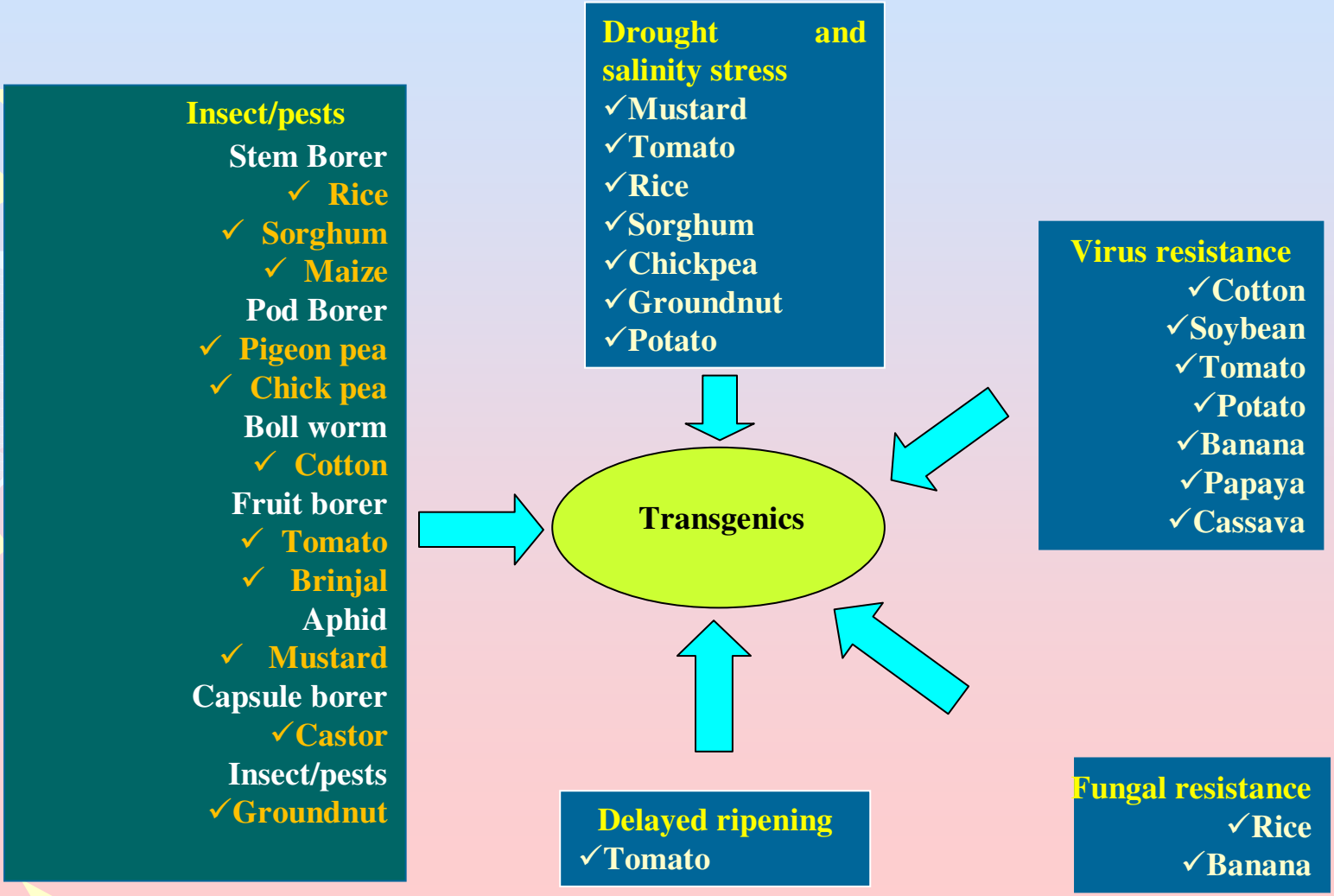
Source: Indian GMO Research Information System (IGMORIS)
www.igmoris.nic.in

Transgenic Development

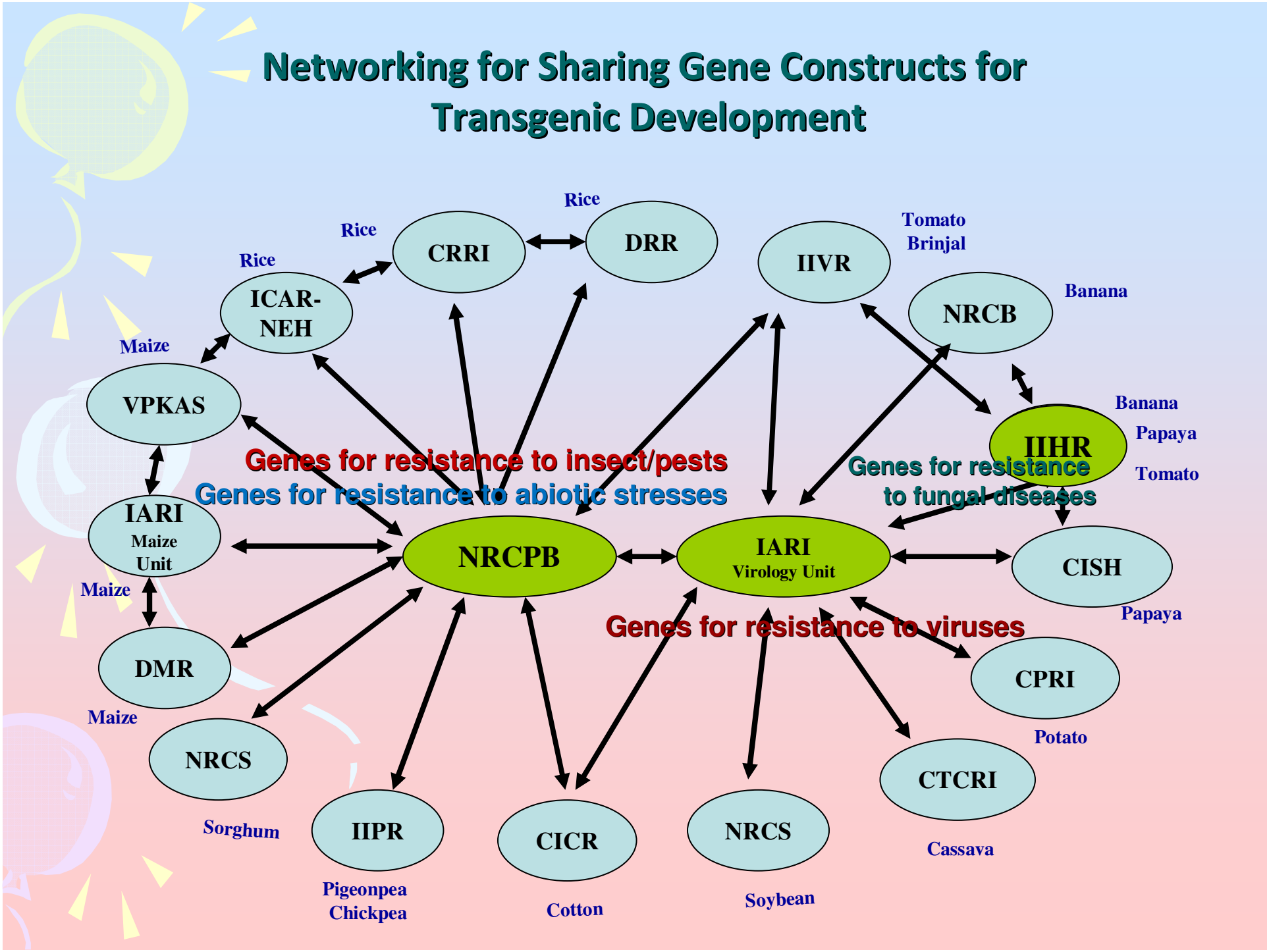
Indian Council of Agricultural Research, New Delhi



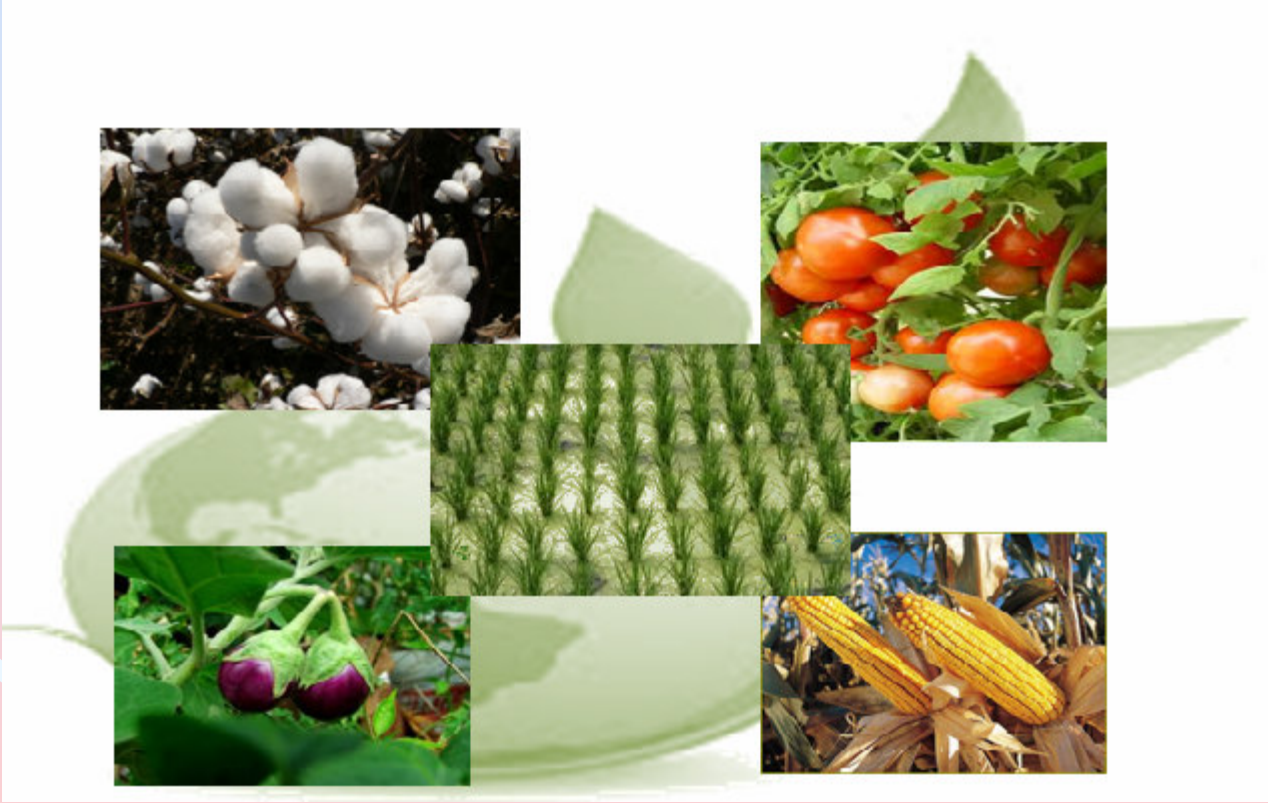
Target Crops and Traits in Network on Transgenics



Networking for Sharing Gene Constructs for Transgenic Development



GM Crops under Field Trials in India 2006-2011



Source: Indian GMO Research Information System **(IGMORIS)**
www.igmoris.nic.in

GM crops under Field Trials 2006

Sr.No.	Crop	Institute/Company Name	Gene/Event
1.	**Brinjal**	IARI, New Delhi Sungro Seeds Ltd, New Delhi Mahyco, Mumbai	*cry1Aa, cry1Aabc* *cry1Ac* *cry1Ac*
2.	**Cabbage**	M/s. Nunhems India Pvt. Ltd., Gurgaon	*cry1Ba , cry1Ca*
3.	**Castor**	Directorate of Oilseeds Research, Hyderabad	*cry1Aa, cry1Ec*
4.	**Cauliflower**	Sungro Seeds Ltd, New Delhi, M/s. Nunhems India Pvt. Ltd., Gurgaon	*cry1Ac, cry1Ba, cry1Ca* *cry1Ac, cry1Ba, cry1Ca*
5.	**Maize**	Monsanto, Mumbai	*cry1Ab/**MON810***
6.	**Groundnut**	ICRISAT, Hyderabad	*Rchit*

GM Crops under Field Trials 2006

7.	**Okra**	Mahyco, Mumbai	*cry1Ac, cry2Ab*
8.	**Potato**	Central Potato Research Institute,Shimla	*RB gene/**for Late Blight***
9.	**Rice**	IARI, New Delhi Tamil Nadu Agricultural University Mahyco, Mumbai	*cry1B-cry1Aa fusion gene* *rice chitinase (chi11) or tobacco osmotin gene* *cry1Ac, cry2Ab*
10.	**Tomato**	IARI, New Delhi Mahyco, Mumbai	*antisense replicase gene of tomato leaf curl virus* *cry1Ac*

Contd....

GM crops under Field Trials 2007

Sr.No.	Crop	Institute/Company Name	Gene/Event
1.	**Rice**	MAHYCO	*cry1Ac*
2.	**Okra**	MAHYCO	*cry1Ac*
3.	**Cotton**	MAHYCO	*stacked cry1Ac, cry2Ab/**Event 15985** CP4EPSPS/**Mon 88913***
4.	**Brinjal**	MAHYCO University of Agricultural Sciences Sungro Seeds Research Ltd. Tamil Nadu Agricultural University	*cry 1 Ac* *cry 1 Ac* *cry 1 Ac* *cry 1 Ac*

GM crops under Field Trials 2008

Sr.No.	Crop	Institute/Company Name	Gene/Event/Trait
1.	**Cauliflower**	Sungro Seeds Research Ltd.	*cry1Ac*
2.	**Cotton** 	Dow AgroSciences India Pvt. Ltd. JK Agri Genetics Ltd MAHYCO Metahelix Life Sciences Central Institute for Cotton Research	*cry1Ac, Cry1F /**Wide Strike*** ***Event 3006-210 /Event 281-24-236*** *cry1Ac/**Event-1*** *cry1EC/**Event-24*** *cry1Ac &cry2Ab/ **MON 15985*** *CP4EPSPS/**MON88913*** *cry1C/**MLS9124*** *cry 1Ac*
3.	**Rice**	Bayer Bioscience Pvt. Ltd.	*cry 1 Ab, cry 1Ca & bar*
4.	**Tomato**	Avesthagen Ltd.	*NAD9/**Increased Lcopene content***
5.	**Maize**	Monsanto India Ltd.	Stacked *cry2Ab2* & *cryA.105/**MON89034*** *CP4EPSPS/**NK603***

GM Crops under Field Trials 2009

Sr.No.	Crop	Institute/Company Name	Gene/Event/Trait
1.	**Brinjal**	Bejo Sheetal Seeds Pvt. Ltd. University of Agricultural Sciences	*cry1Fa1*/***Event 142*** *cry1Ac*
2.	**Cabbage & Cauliflower**	Nunhems India Pvt. Ltd	*cry1Ba, cry1Ca , bar*
3.	**Cotton**	JK Agrigenetics Ltd Dow Agrosciences Central Institute for Cotton Research	*cry1Ac*/***Event1*** *cry1EC*/***Event142*** *cry1 Ac & cry1F*/***Widestrike*** ***Event 3006-210-23 and Event 281-24236*** *ACP, SCP, AReP*/***Virus resistance***
4.	**Potato**	Central Potato Research Institute	*GA20 Oxidase1*/**Dwarf Potato**
5.	**Maize** 	Monsanto India Ltd. Pioneer Overseas Corporation Dow Agrosciences India Pvt. Ltd.	*cry2Ab2, cryA.105*/***MON89034*** *CP4EPSPS*/***NK603*** *cry1F&CP4EPSPS*/***TC1507xNK603*** *Cry1F*/**Event TC1507**

Contd....

GM Crops under Field Trials 2009

6.	**RRF cotton**	Maharashtra Hybrid Seeds	*cry1Ac & cry2Ab/**MON 15985*** *CP4EPSPS/**MON88913***
7.	**Rice**	Bayer Bioscience Pvt. Ltd. Maharashtra Hybrid Seeds	*cry 1Ab, cry 1Ca & bar* *cry2Ab*
8.	**Groundnut**	ICRISAT, Hyderabad	*Coat protein gene/**Virus resistance*** *Rchit gene/**resistance againt Aspergillus*** *DREB1A gene/**Abiotic tolerence/Drought resistance***
9.	**Chickpea**	ICRISAT, Hyderabad NRC for Plant Biotechnology	*DREB1A gene/**Abiotic tolerence/Drought resistance*** *cry2Aa/**Insect resistance***
10.	**Sorghum**	National Research Centre for Sorghum	*cry1b/**Insect resistance***

GM Crops under Field Trials 2010

Sr.No.	Crop	Institute/Company Name	*Gene/Trait/Event*
1.	**Watermelon**	Indian Institute of Horticultural Research, Bangalore	*Water Bud Necrosis Virus /Virus resistance*
2.	**Papaya**	Indian Institute of Horticultural Research, Bangalore	*PRSV cpgene/**Virus resistance***
3.	**Cauliflower and Cabbage**	Nunhems India Pvt Ltd	*cry1Ba, cry1Ca, Bar/* ***Insect resistance***
4.	**Sugarcane**	Sugarcane Breeding Institute	*cry1Ab/**Insect resistance***
5.	**Sorghum**	Central Research Institute for Dryland Agriculture, Hyderabad	*mtID gene/ **Abiotic tolerance/drought resistance***
6.	**Maize**	Pioneer Overseas Corporation Dow AgroSciences India Pvt. Ltd. Syngenta Biosciences Pvt. Ltd Monsanto India Ltd.	*cry1F &Pat, CP4EPSPS* ***Insect resistance and herbicidetolerence/TC1507XNK603/DAS-01507-1xMon-00603-6*** *Cry1 F /**Event TC 10507*** *Cry1Ab/**Event Bt 11*** *stacked cry2Ab2, cry1A.105 /**Event MON 89034*** *CP4EPSPS /**Event NK 603*** ***Event 89034XNK603***

7.	**Cotton**	Dow AgroSciences India Pvt. Ltd. JK Agri Genetics Ltd. Central Institute for Cotton Research Krishidhan Seeds Ltd	*cry1Ac & cry1F/**Wide Strike*** *cry1Ac/**Event-1*** *cry1EC/**Event-24*** *cry1AC* *cry1Ac, cry1F and cry1EC/* ***MIR Cotton1-131***
8.	**Glytol cotton**	Bayer Bioscience Pvt. Ltd	*2mEPSPS*
9.	**Brinjal**	Indian Institute of Vegetable Research	*cry1Ac*
10.	**Mustard**	National Research Centre on Plant Biotechnology, New Delhi Uni. of Delhi South Campus, N.Delhi	*osmotin* *barnase, barsar and bar*
11.	**Rubber**	Rubber Research Institute of India	*manganese superoxide dismutase gene (cDNA)/Abiotic Stress Tolerance*
12.	**Groundnut**	ICRISAT, Hyderbad University of Agricultural Sciences,GKVK Campus	*chitinase gene* *over expressed DREB1B*

Contd....

13.	**Hybrid rice SPT maintainer**	E.I. DuPont India Pvt. Ltd	*Os-Msca1, ZM-AA1, Os-MSCA1, DsRED2*
14.	**Rice**	Bayer Bioscience Pvt Ltd. Metahelix LifeSciences Pvt. Ltd. BASF India Ltd	*cry1Ab, cry1Ca & bar* *cry1Ac and cry1Ab* *RAP5-11*
15.	**Tomato**	Indian Institute of Horticultural Research, Bangalore Indian Institute of Vegetable Research,Varanasi National Research Centre on Plant Biotechnology, New Delhi	*Virus resistance gene* *cry1Ac* *Antisense ACC synthase 2*

GM crops under field trials 2011

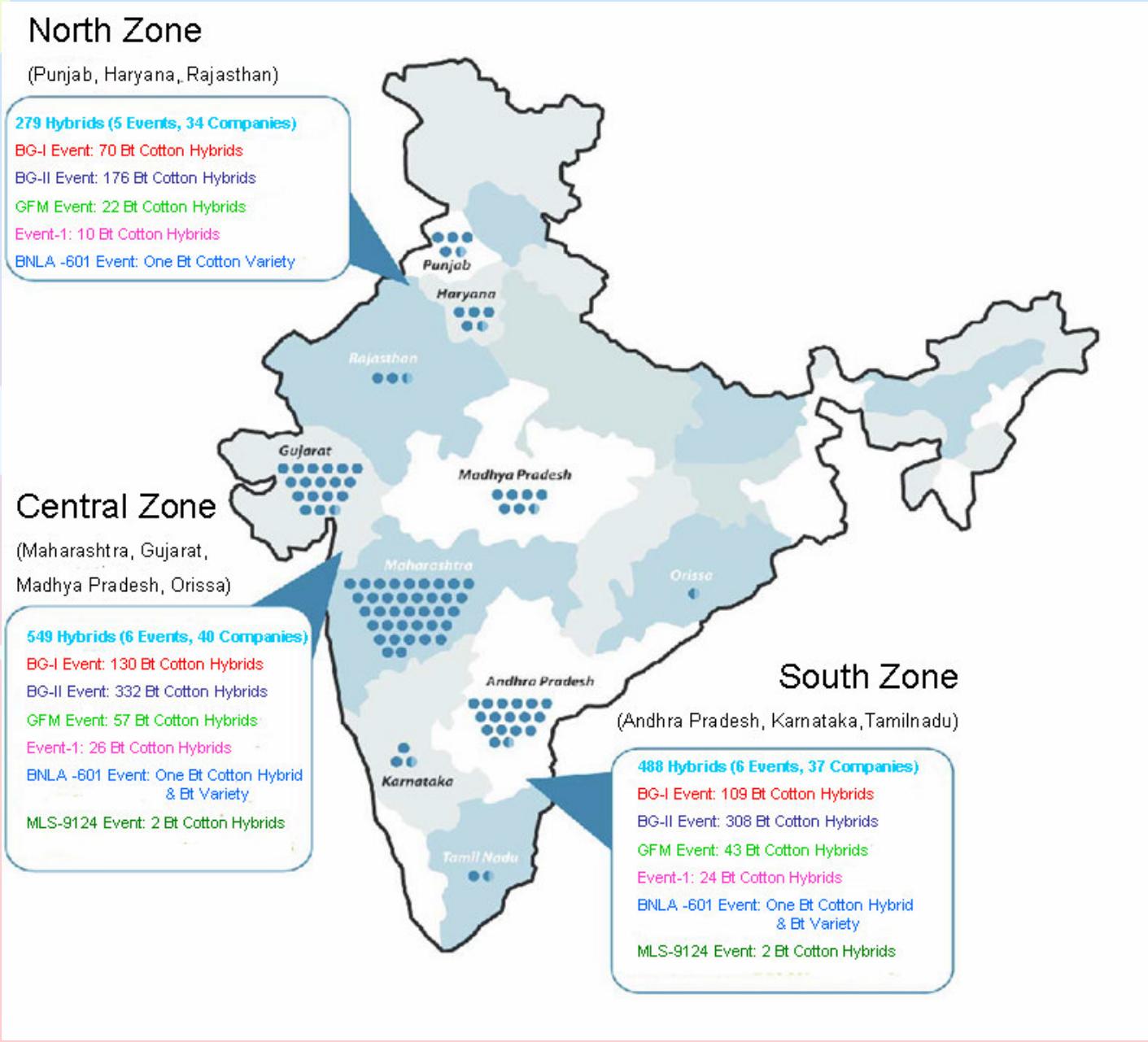
Sr.No.	Crop	Institute/Company Name	Gene/ Trait/Event
1.	**Rubber**	Rubber Research Institute of India	*manganese superoxide dismutase gene (cDNA)/* ***Abiotic Tolerance***
2.	**Sorghum**	Directorate of Sorghum Research	*cry1B /**Insect Resistance***
3.	**Ground nut**	ICRISAT	*Chitinase rice DREB1A (Chitinase)/**Fungal Resitance***
4.	**Rice**	E.I. Dupont India Pvt. Ltd	*Zm-AA1, Os-Msca1, DsRed2/* ***Male Sterile female inbread ricelines***
		BASF India Limited	*RPD5 -11*
		Bayer BioSciences Pvt. Ltd.	*cry1Ab, cry1Ca, bar/* ***Insect resistance***
		Department of Botany, University of Calcutta	*Ferritin/**Stress tolerance***

Commercially Released Hybrids/Variety of Six events of Bt cotton in India from 2002-2011

Event	Developer	Year of Approval	Total Hybrids
BG-I	Mahyco/Monsanto	2002	215
BG-II	Mahyco/Monsanto	2006	528
Event-I	JK Agri-Genetics	2006	41
GFM Event	Nath Seeds	2006	96
BNLA-601	CICR (ICAR & UAS, Dharwad	2008	2
MLS-9124	Metahelix Life Sciences	2009	2

Source: ISAAA, 2011

Bt Cotton Cultivation in India



GM Detection Work in India

Cooperation and Collaboration

भाकृअनुप
ICAR

हर कदम, हर डगर
किसानों का हमसफर
भारतीय कृषि अनुसंधान परिषद

Agrisearch with a human touch

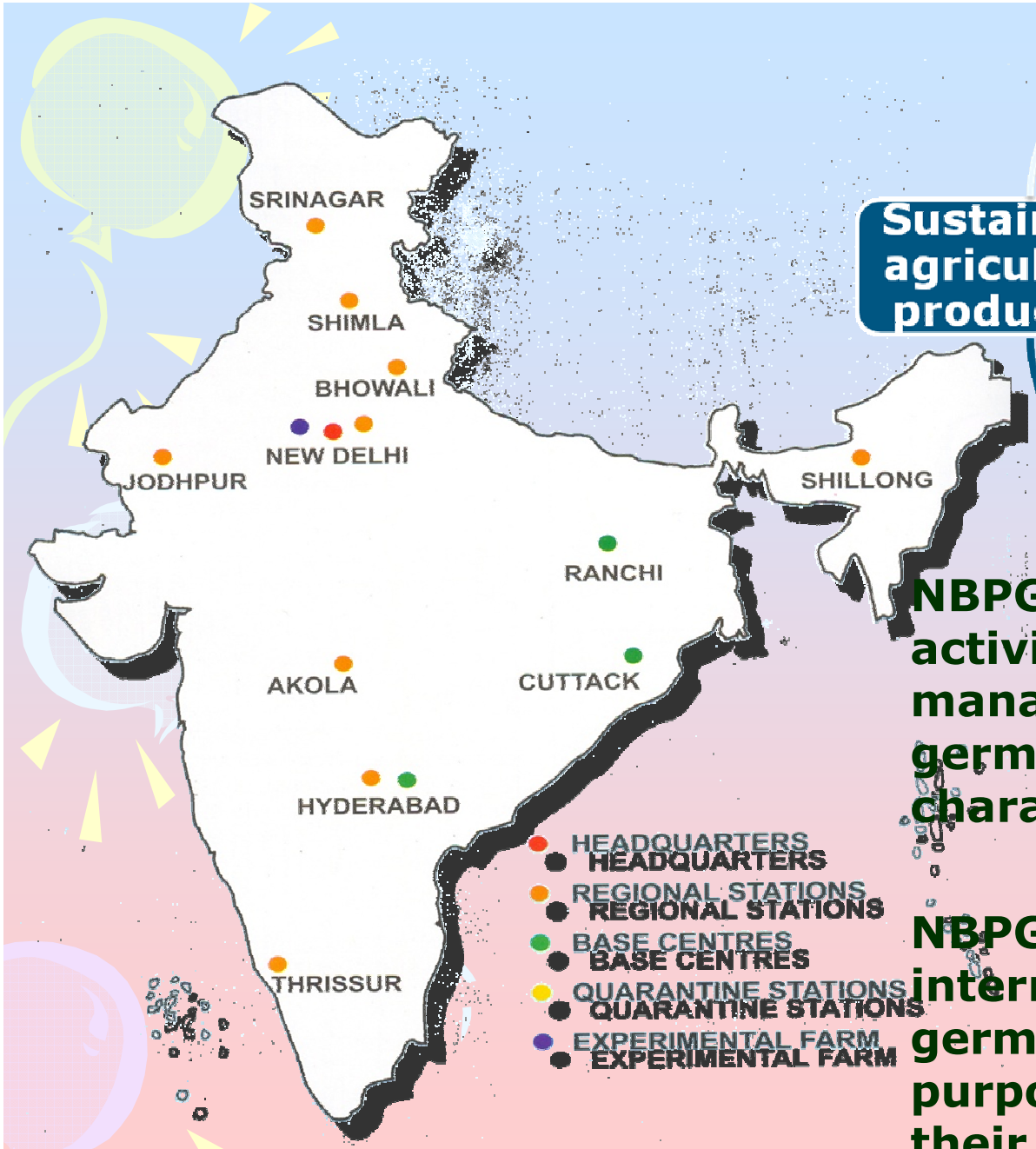
रा पा आ सं ब्यूरो
NBPGR

National Bureau of Plant Genetic Resources

Indian Council of Agricultural Research





Sustainable agricultural production

PGR management

NBPGR is involved in all activities of PGR management by undertaking germplasm collection, characterization, evaluation

NBPGR facilitates international exchange of germplasm for research purposes and carries out their quarantine processing

DNA-based GM detection work at NBPGR

National Bureau of Plant Genetic Resources

Nodal Agency for:

Import Permit

Quarantine Processing

Issue of Phytosanitary Certificate

For Germplasm/ Transgenic Planting Materials

DBT Import Clearance

- **Para 4**

  applicant to certify to NBPGR material being imported conform to the description given in the import clearance

  NBPGR to retain 5% of the seed in the safe custody

- **Para 5**

  supplier to certify that the imported transgenic material contains transgenes conforming to those described in the permission no embryo-genesis deactivator (terminator) gene

Sponsored Projects on GM Detection/Biosafety

Department of Biotechnology, Govt. of India

- National Containment/Quarantine Facility for the Testing of Transgenic Planting Material- **1999-ongoing**
- PCR-based Testing of Transgenic Planting material- **2002-2007**
- Referral Centre for Molecular Diagnosis of Transgenic Planting Material-**2007-ongoing**
- Multi-target System for GM Detection and Quantification in GM Food Crops -**2011-ongoing**

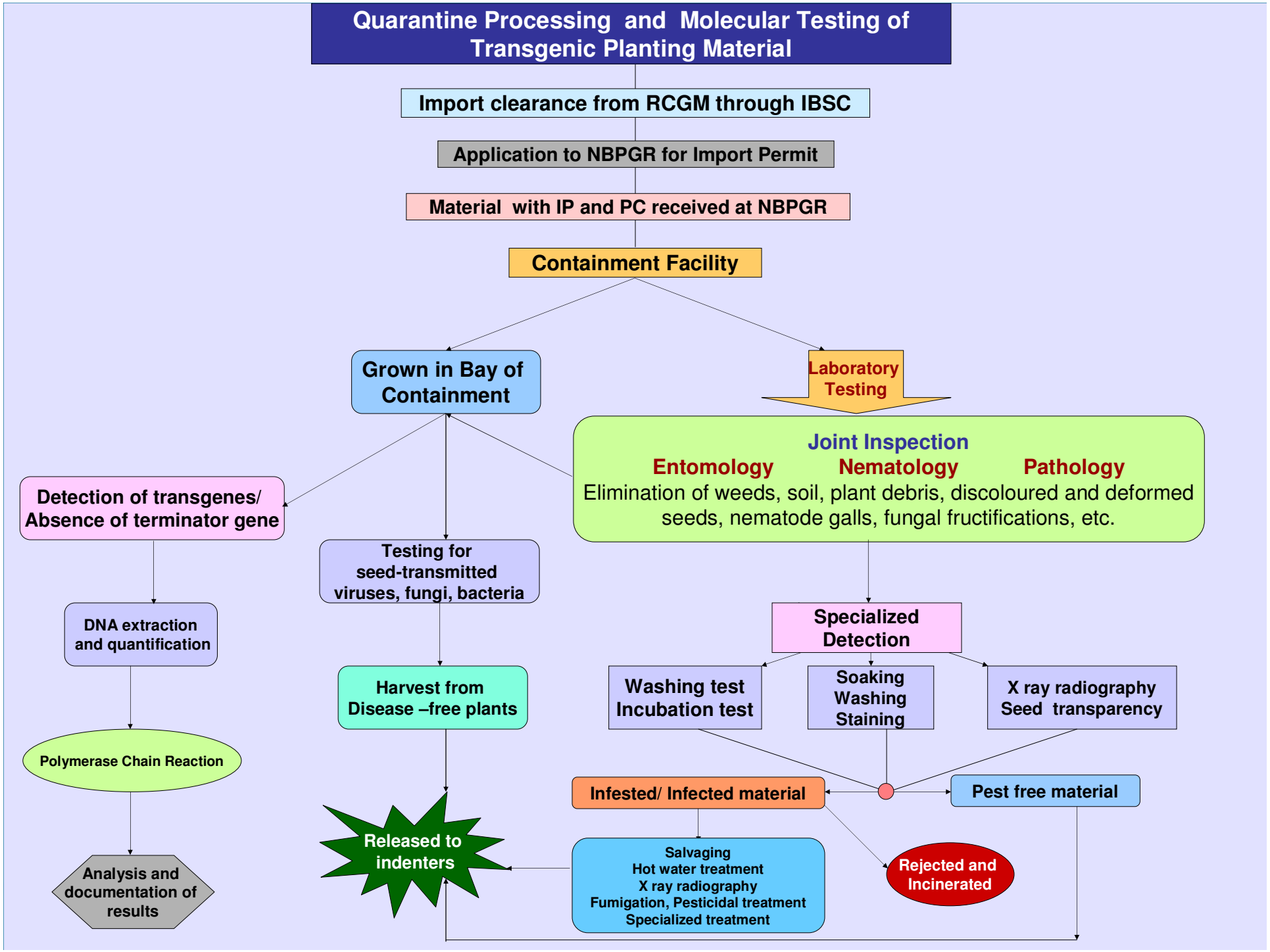
GEF-World Bank, MoEF, Govt. of India

- Implementation of Cartagena Protocol on Biosafety in India

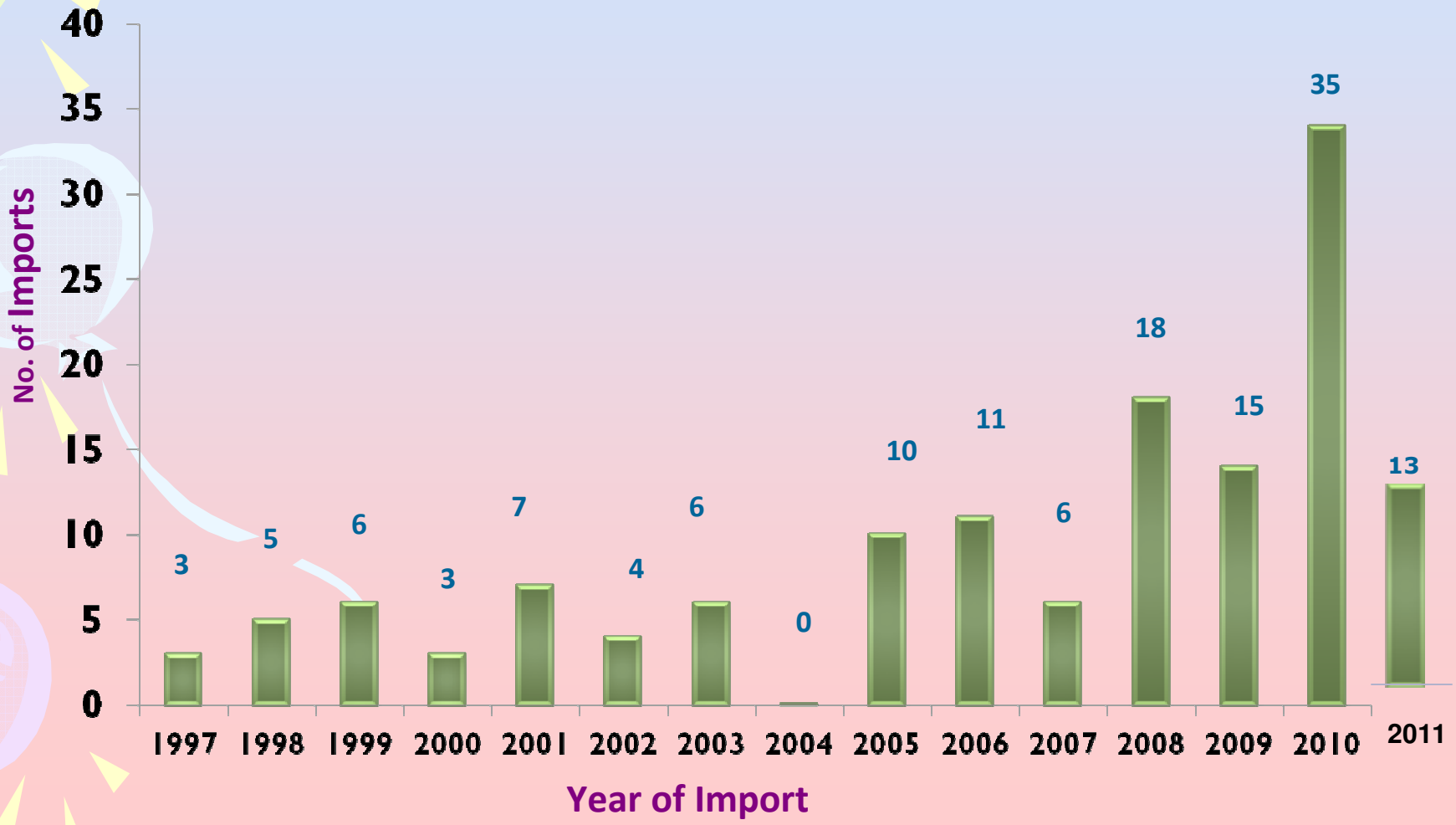
-2004-2007 Phase I

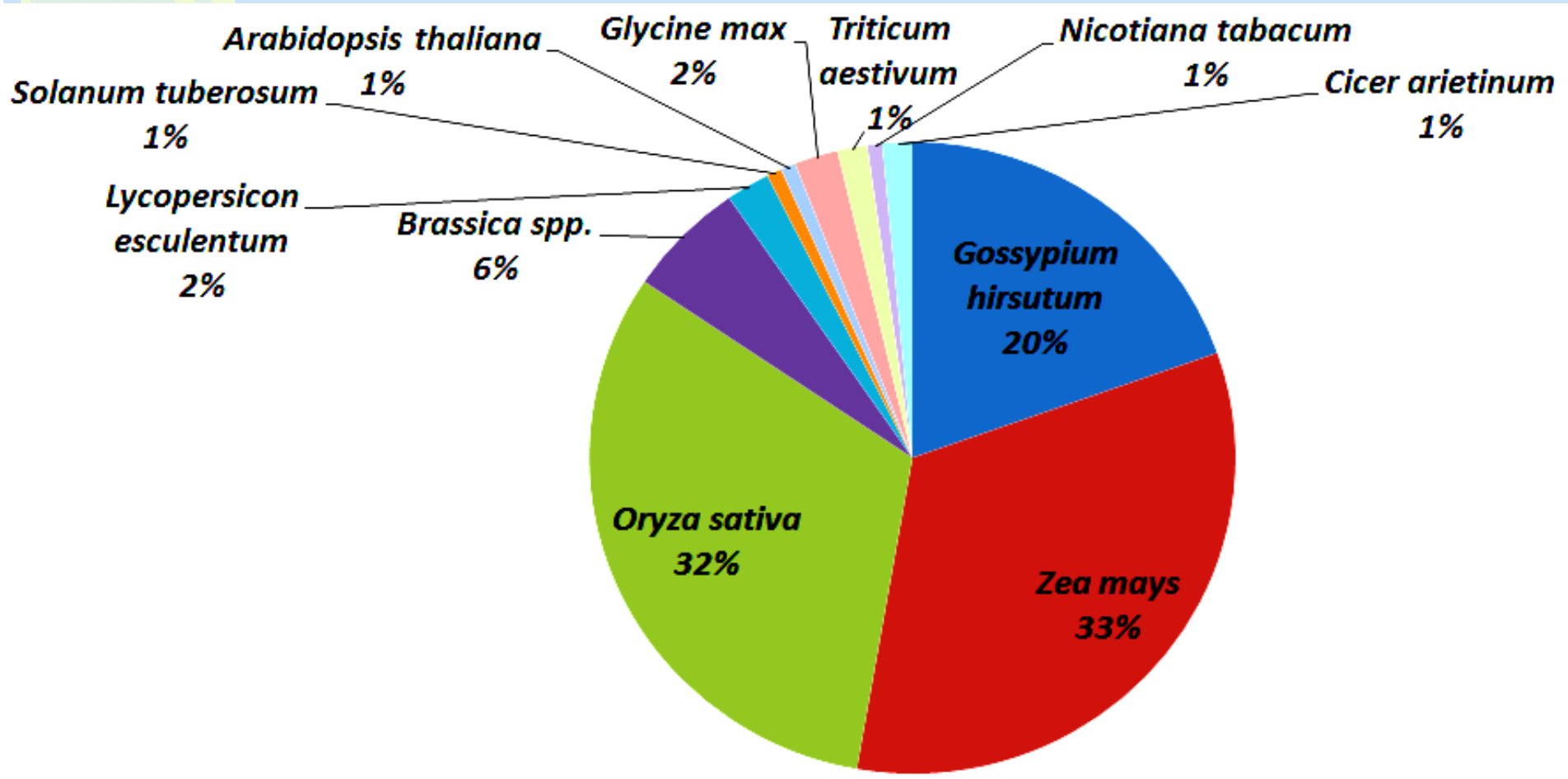
GEF-UNEP, MoEF Starting in near future Phase II

Facilities at NBPGR for GM detection


Quarantine Processing and Molecular Testing of Transgenic Planting Material
Import clearance from RCGM through IBSC
Application to NBPGR for Import Permit
Material with IP and PC received at NBPGR
Containment Facility
Grown in Bay of Containment
Laboratory Testing
Joint Inspection
Entomology
Nematology
Pathology
Elimination of weeds, soil, plant debris, discoloured and deformed seeds, nematode galls, fungal fructifications, etc.
Detection of transgenes/ Absence of terminator gene
Testing for seed-transmitted viruses, fungi, bacteria
DNA extraction and quantification
Specialized Detection
Harvest from Disease –free plants
Washing test Incubation test
Soaking Washing Staining
X ray radiography Seed transparency
Polymerase Chain Reaction
Infested/ Infected material
Pest free material
Released to indenters
Salvaging
Hot water treatment
X ray radiography
Fumigation, Pesticidal treatment
Specialized treatment
Rejected and Incinerated
Analysis and documentation of results

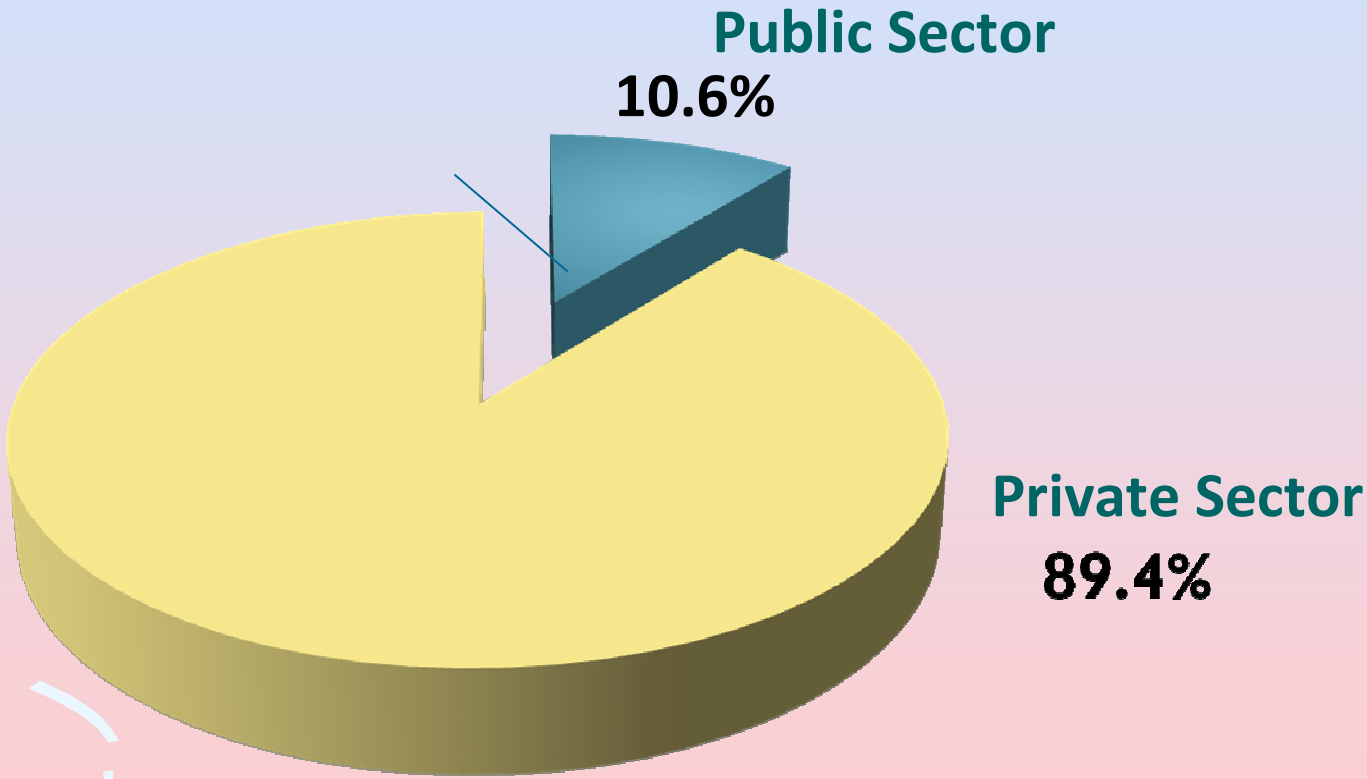
Imported Transgenic Planting Material (1997-2011)
Total No. of imports: 145 constituting 2614 accessions



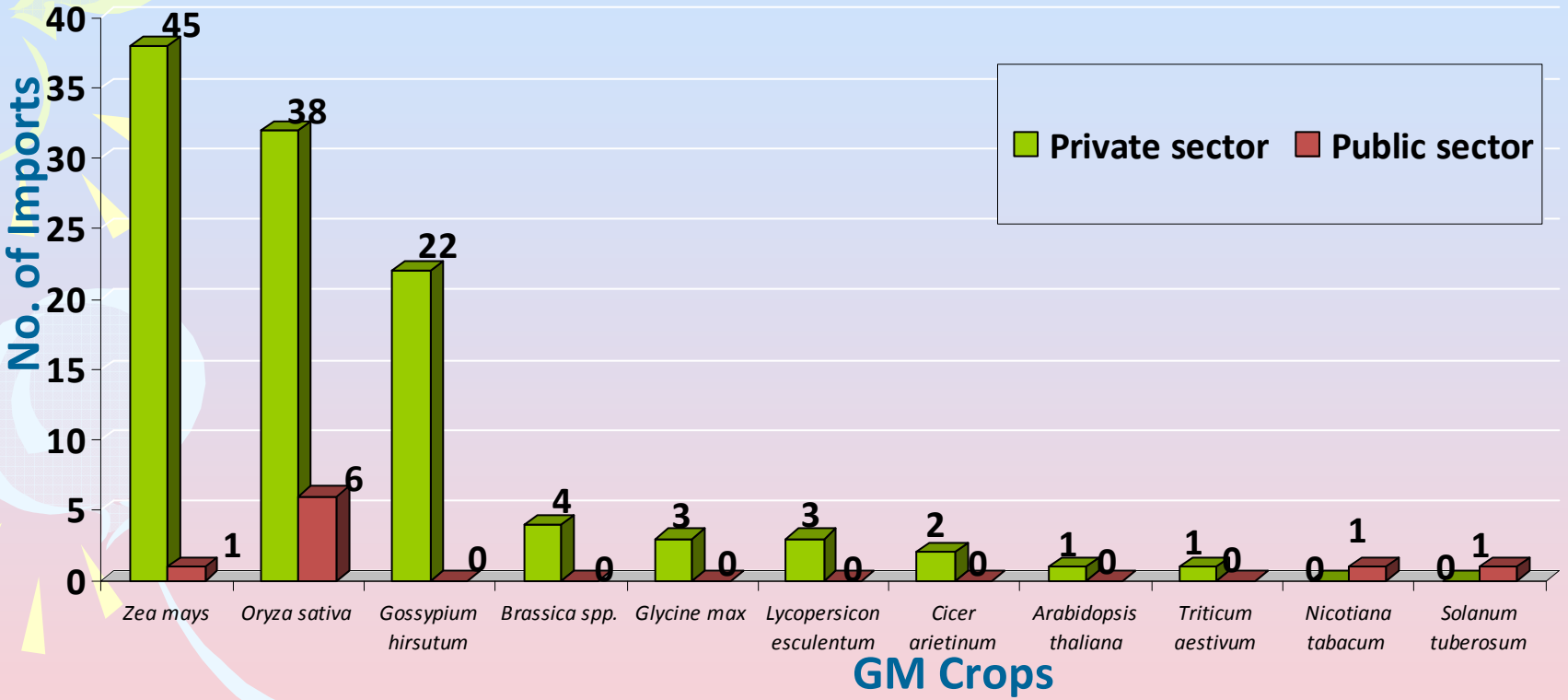


Crop-wise Import of Transgenic Planting Material from 1997-2011

GM Imports (145) by Public and Private Sector



Crop-wise (no.) Imports by Public and Private Sector



Imports by Public Sector

Zea mays: UAS, GKVK campus, Bangalore

Oryza sativa: IARI, New Delhi (2), DRR, Rajendranagar, Hyderabad (2), TNAU, Tamil Nadu (1), Rice Research Station, WB(1)

Nicotiana tabacum: University of Hyderabad, Hyderabad

Solanum tuberosum: CPRI, Shimla

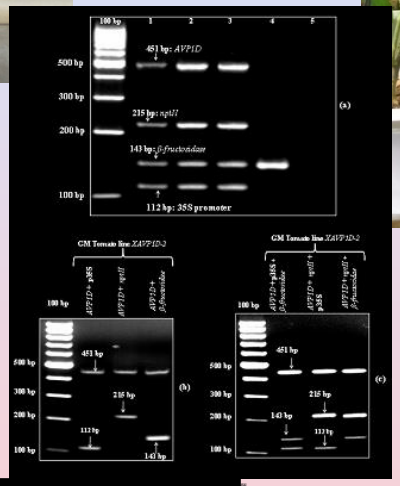
Imported Transgenic Planting Material (1997-till date)
Total No. of imports: 145 constituting 2614 accessions

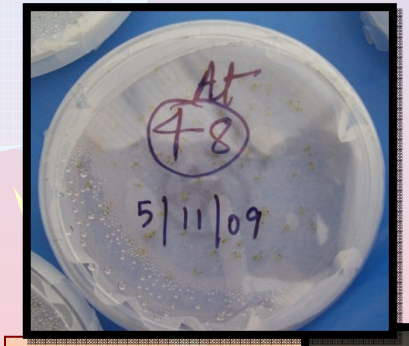
Crop & No. of Imports	Transgenes	Traits	Country of Import
Zea mays (50)	*cry1Ab, cry1A.105, cry2Ab2, cry1F* *gat, cp4epsps, mepsps* *Gus & control elements*	Insect resistance Herbicide tolerance	USA, South Africa, Philippines
Oryza sativa (48)	*cry1Ac, cry1Ab, cry1Ca, cry19C, GFM-cry1A, cry2A* *AmA1, ferritin, crtI, lcy* *Basta, cp4epsps, bar* *Xa-21* *HAS, ScFv, AFP-AG*	Insect resistance High nutritional quality Herbicide tolerance Bacterial pathogen resistance Nematode resistance	USA, Belgium, Philippines, UK, Switzerland, Philippines China
Gossypium hirsutum (26)	*cry1Ac, cry2Ab, cry1Ab-cry1Ac, cp4epsps, cry1F, vip3A, cry2Ae, cry1Ab* *Cp4epsps* *35S-rolA, B, C & Mannosyl transferase* *At ANP1, AtSOS2, At A-20, At CBF3, At SOS1*	Insect resistance Herbicide tolerance Drought tolerance Salinity and drought tolerance	China, USA

Brassica spp. (8)	*cry9C, cry1Ba, cry1Ca* *Barnase, barstar, bar* *Osmades-1*	Insect resistance Male sterility and restoration of male fertility & glufosinate ammonium herbicide resistance Reduced apical dominance	Belgium, Netherlands, Australia
Lycopersicon esculentum (3)	*AVP1* *Arg*	Increased salt and drought tolerance Insect resistance	USA
Glycine max (3)	*Cp4epsps*	Herbicide tolerance	USA
Triticum aestivum (2)	*HAS, ScFv, AFP-AG* *Cp4epsps*	Nematode resistance Herbicide tolerance	Germany, USA
Cicer arietinum (2)	*Bean-alpha amylase inhibitor*	Insect resistance	Australia, Scotland
Arabidopsis thaliana (2)	*35S promoter*	Regulatory element	USA
Nicotiana tabacum (1)	*Alternate oxidase*		Canada
Solanum tuberosum (1)	*RB*	Late blight resistance	USA

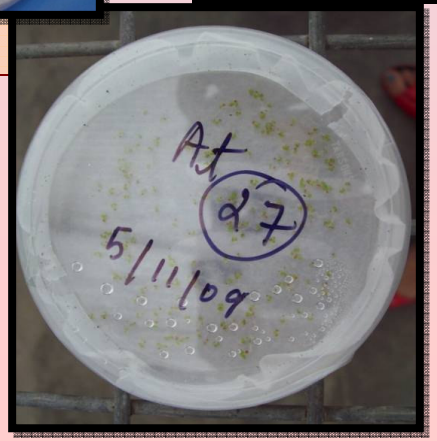
Molecular testing of all 2614 imported transgenic lines of 145 imports

GM tomato





GM Arabidopsis



Bt cotton events

Quadruplex PCR in imported Tomato line *XAVP1D-2* ; (a) for the detection of *AVP1D* gene, *nptII* marker gene, *CaMV* 35S promoter and exon 7 of *β-fructosidase* as endogenous gene, Lanes 1-2: GM tomato line *XAVP1D-2*, Lane 3: Non-GM tomato, Lane 4: Water control; (b) Duplex PCR; (c) Triplex PCR

Department of Biotechnology, Govt. Of India

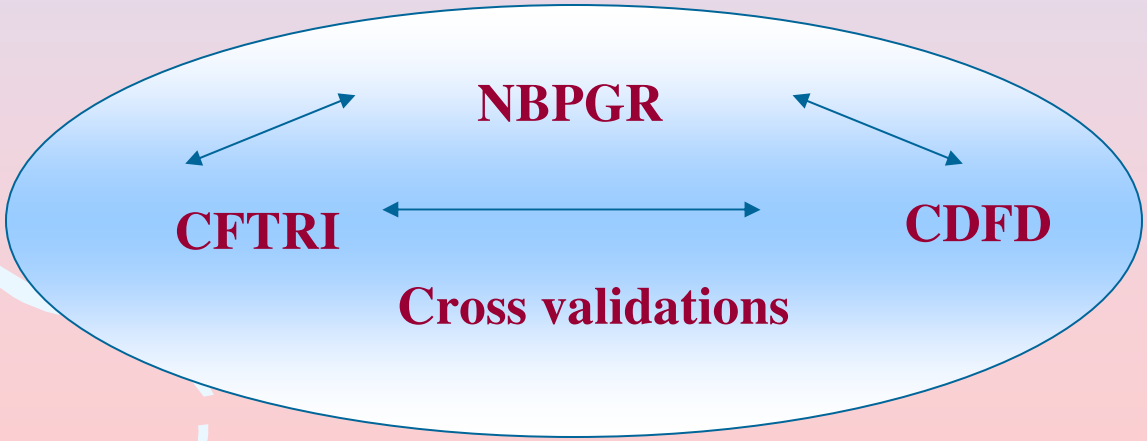
Central Food Technology Research Institute, Mysore

Centre for DNA Fingerprinting and Diagnostics, Hyderabad

National Bureau of Plant Genetic Resources, New Delhi

National Institute of Nutrition, Hyderabad

Industrial Toxicology Research Center, Lucknow



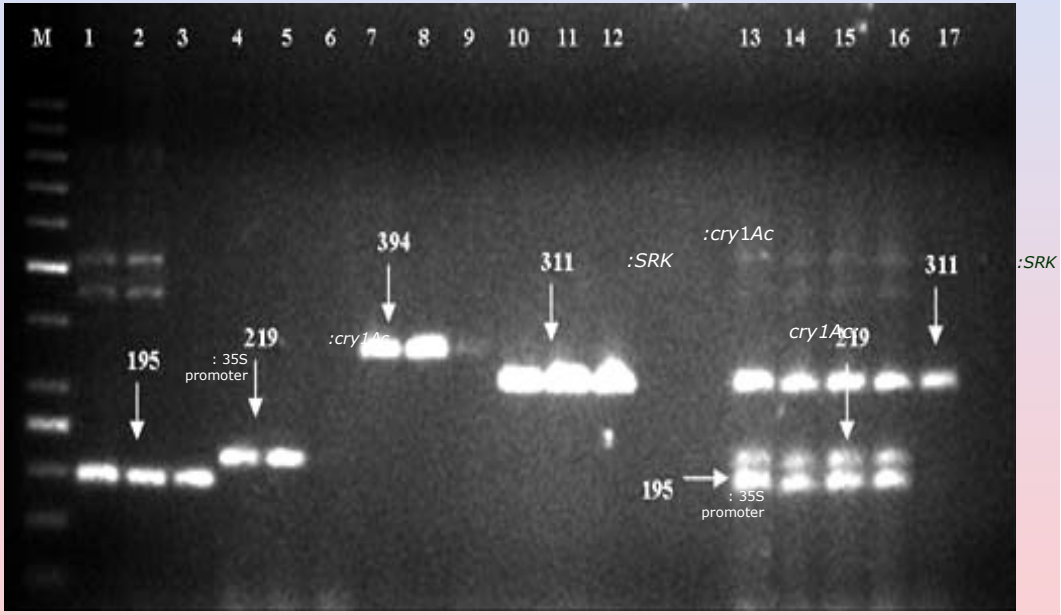
Three Rounds of Cross-validations were successfully completed

2002-2007

Identification, Standardization and Validation of Endogenous Genes in GM Crops

Crops	Endogenous genes
Cotton	*Sad1 (Steroyl acyl desaturase), fs-ACP (Fibre-specific acyl carrier protein)*
Rice	*SPS (Sucrose phosphate synthase), TubA (α-tubulin A)*
Maize	*Zein, Adh1, hmg*
Tomato	*LAT52 (Late anther tomato), exon 7 of β-fructosidase*
Brinjal	*exon 7 of β-fructosidase*
Cauliflower	*SRK (S-locus receptor kinase)*
Mustard	*HMG1*
Potato	*exon 7 of β-fructosidase, ST-LS1*

Multiplex PCR in Bt cauliflower and validation of endogenous gene in Brassicaceae family



Source: Randhawa GJ *et al.* (2008) Molecular Characterization of Bt Cauliflower with Multiplex PCR and Validation of Endogenous Reference Gene in Brassicaceae Family. *Current Science*. 95, No.12 :1729-31

Initial Screening through Simplex/multiplex PCR-based Amplification of Marker and Reporter Genes for Initial Screening

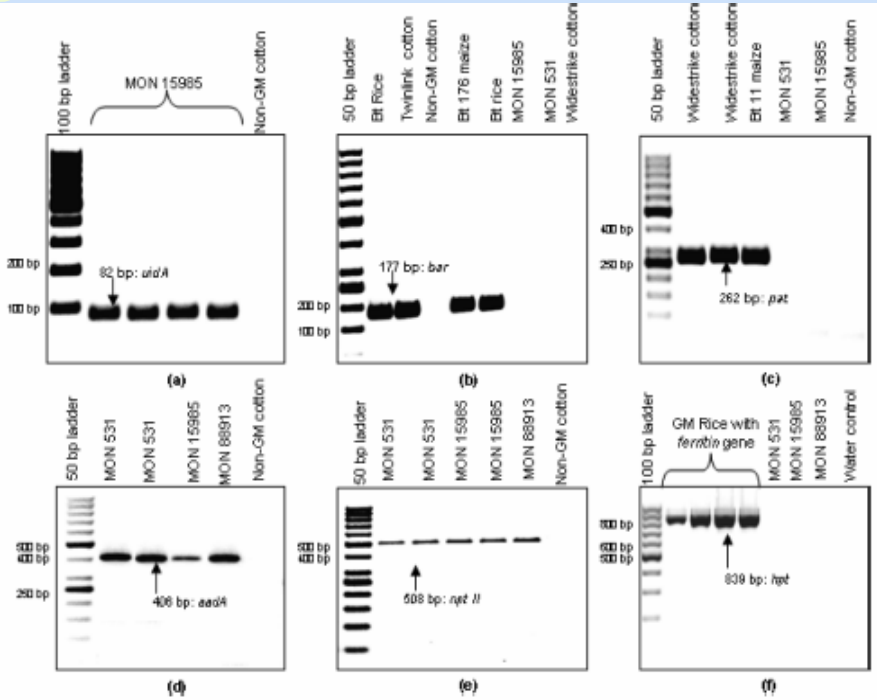


Figure 1. Simplex PCR amplicons in different transgenic seed materials for their respective selectable marker genes using the specific primer pairs for the detection of (a) *uidA*; (b) *bar*; (c) *pat*; (d) *aadA*; (e) *npt II*; and (f) *hpt* genes.

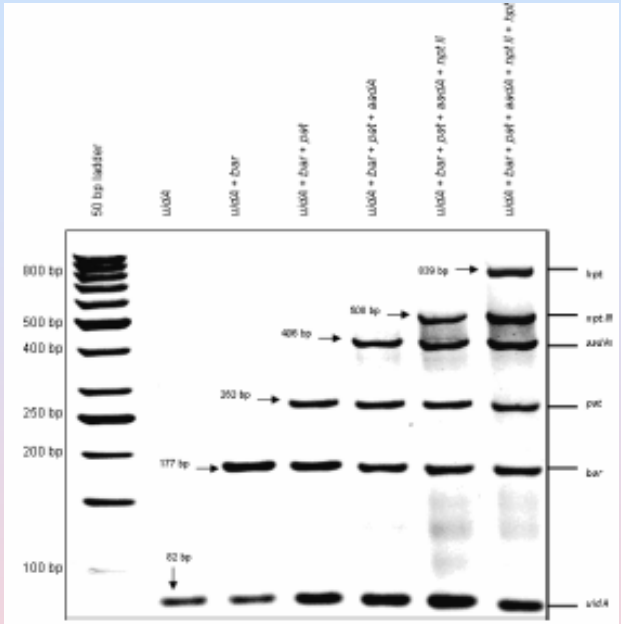
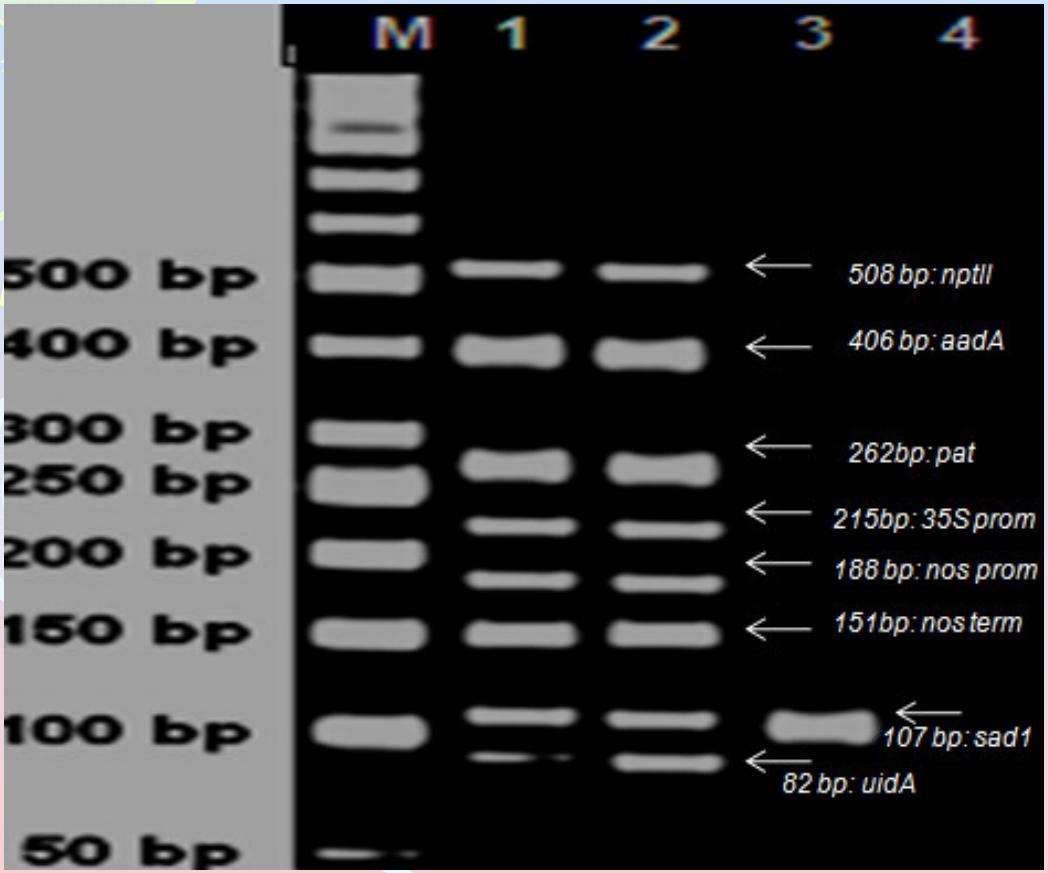


Figure 2. Multiplex PCR assay for testing of primer interference using equivalent DNA mix of six different GM events, i.e., MON 531 of cotton, MON 15985 of cotton, Widestrike cotton, Bt rice, GM rice with the ferritin gene and Bt176 of maize.

Simultaneous amplification of six commonly used marker genes *viz*., *nptII, aadA, bar, pat, hpt* and *uidA*

Randhawa G.J. *et.al* (2009) Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for screening of genetically modified crops. *J. Agri. Food Chem*. 57 (12): 5167-5172.

Combo Octaplex PCR for screening of GM Crops



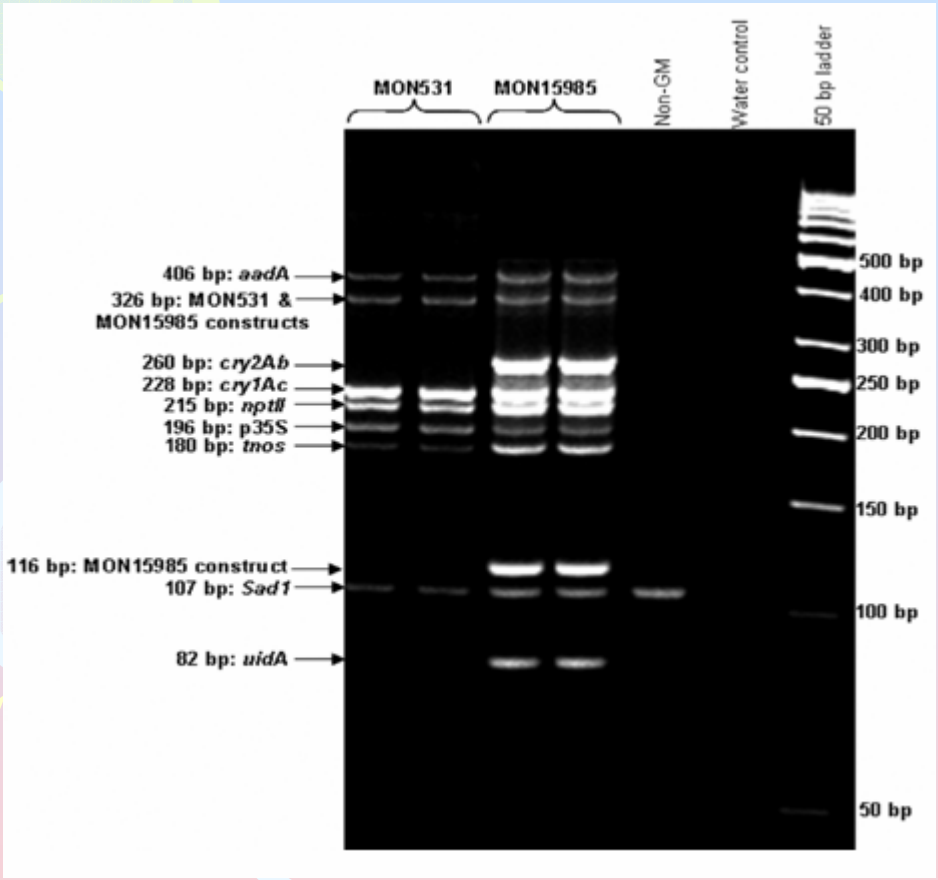
Simultaneous amplification of seven control elements viz., 35S promoter, *nos* terminator, *nos* promoter, marker genes; *nptII*, *aadA*, *pat* and *uidA* and endogenous gene *sad1*

Lane M: 50 bp ladder; Lane 1 & 2: mixture of MON531, MON15985, GFM cry1A and Widestrike events of GM cotton (covering all the seven regulatory elements used for study)

Identification of specific transgene by simplex PCR /multiplex PCR
Development of qualitative and quantitative PCR assays

Event	Multiplex PCR	Transgenes + reference gene involved
Bt cotton MON 531	Heptaplex	*fs-ACP + cry1Ac + 35S promoter + nos term. + nptII + aadA + cry1Ac construct*
Bt cotton MON 15985	Decaplex	*fs-ACP + cry1Ac + cry2Ab + 35S promoter + nos term. + nptII + aadA + uidA + cry1Ac construct + cry2Ab construct*
Bt Rice	Triplex	*cry1Ac, nptII + α-tubulin*
Bt Brinjal	Quadraplex	*cry1Ac, caMV 35S promoter, aadA + β- fructosidase*
Bt Brinjal	Triplex	*cry1Ab, 35S promoter + β- fructosidase*
Bt cauliflower	Triplex	*cry1Ac, 35S promoter + SRK*
Bt Okra	Quadraplex	*cry1Ac, nptII, 35s promoter + chloroplast t-RNAomat*
GM tomato	Quadraplex	*Avp1, nptII, 35S promoter + LAT52,*
GM tomato	Triplex	*Osmotin + 35S promoter + LAT52,*
GM potato	Triplex/ Quadraplex	*RB gene, CaMV 35S promoter, npt II marker + UGPase*
GM potato	Triplex/ Quadraplex	*Ama1 gene, CaMV 35S promoter, nos terminator, nptII + UGPase*
GM potato	Triplex/ Quadraplex	*cry1Ab gene, CaMV 35S promoter, nos , nptII + UGPase*

Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events



Transgene- and construct-specific multiplex PCR for discrimination of two Bt cotton events, i.e., MON531 and MON15985 using primer pairs for *cry1Ac* and *cry2Ab* transgenes, *nptII, aadA*, and *uidA* marker genes, *CaMV* 35S promoter, *nos* terminator, endogenous *Sad1* gene, and specific gene constructs in MON531/MON15895 and MON15985.

Duplex, triplex and quadraplex PCR of GM Potato for improved protein quality

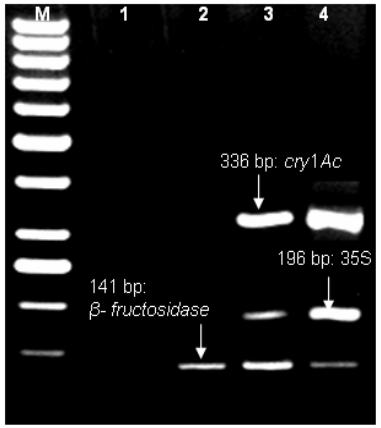
PCR in duplex, triplex and Quadraplex format for Detection of GM Potato with *Ama1* gene
Lane M: 50 bp DNA ladder; Lanes T1, T2, T3,: GM potato with *AmA1* gene; Lane C: Non-GM potato

Source: 1. Randhawa G.J., R Chhabra and M Singh (2010) Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events. *Journal of Agriculture and Food Chemistry* (2010) *58* (18), 9875–9881.

2. Randhawa G.J, Monika Singh & Ruchi Sharma (2009) Duplex, triplex and quadruplex PCR for molecular characterization of genetically modified potato with better protein quality. *Current Science*, 97 (1): 21-23.

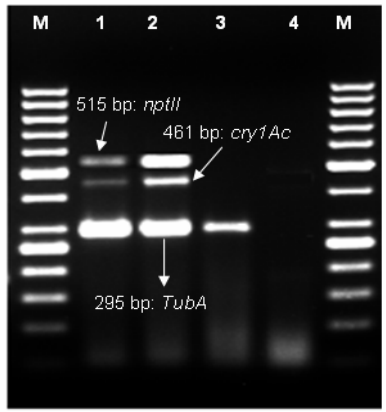
Multiplex PCR assays for detection of *Bt* crops with *cry1Ac* gene

Bt Brinjal



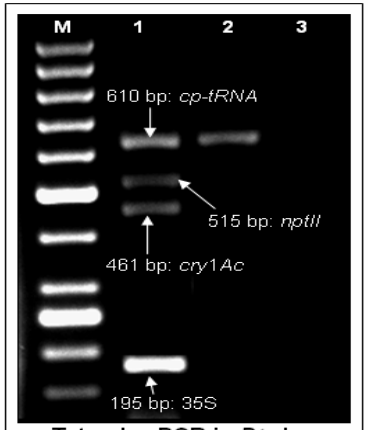
Triplex PCR in *Bt* brinjal with *cry1Ac* gene
1: Water control
2: Non-*Bt* brinjal
3-4: *Bt* brinjal
M: 50bp DNA ladder

Bt Rice



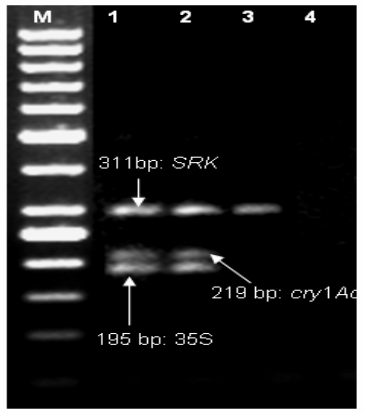
Triplex PCR in *Bt* rice with *cry1Ac* gene
1-2: *Bt* rice
3: Non-*Bt* rice
4: Water control
M: 50bp DNA ladder

Bt Okra



Tetraplex PCR in *Bt* okra with *cry1Ac* gene
1: *Bt* okra
2: Non-*Bt* okra
3: Water control
M: 50bp DNA ladder

Bt cauliflower

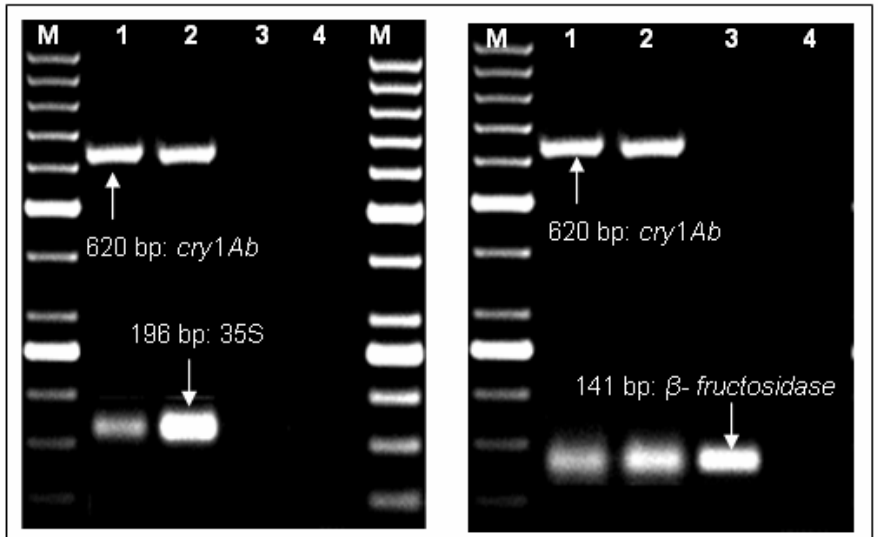


Triplex PCR in *Bt* cauliflower with *cry1Ac* gene
1-2: *Bt* cauliflower
3: Non-*Bt* cauliflower
4: Water control
M: 50bp DNA ladder

Source: Randhawa G.J., M Singh, R Chhabra and R Sharma (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India. *Food Analytical Methods* DOI 10.1007/s12161-010-9126-8

Multiplex PCR assays for detection of *Bt* crops with *cry1Ab* gene

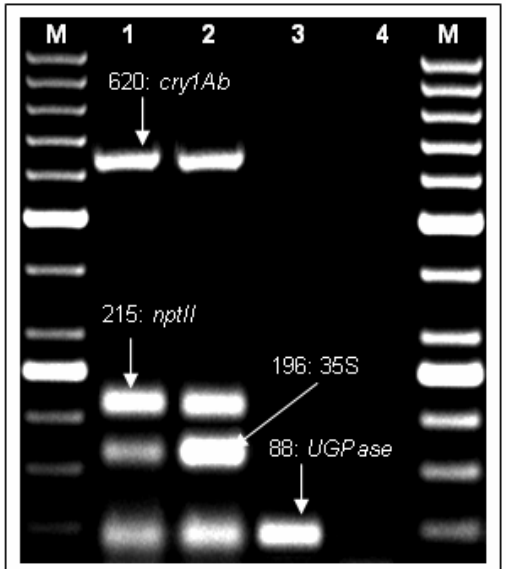
Bt Brinjal



Duplex PCR in *Bt* brinjal with *cry1Ab* gene simultaneously detecting (i) *cry1Ab* and *CaMV* 35S *promoter; and (ii) cry1Ab and endogenous β- fructosidase* gene

1-2: *Bt* brinjal
3: Non-*Bt* brinjal
4: Water control
M: 50bp DNA ladder

Bt Potato



Tetraplex PCR in *Bt* potato with *cry1Ab* gene

1-2: *Bt* potato
3: Non-*Bt* potato
4: Water control
M: 50bp DNA ladder

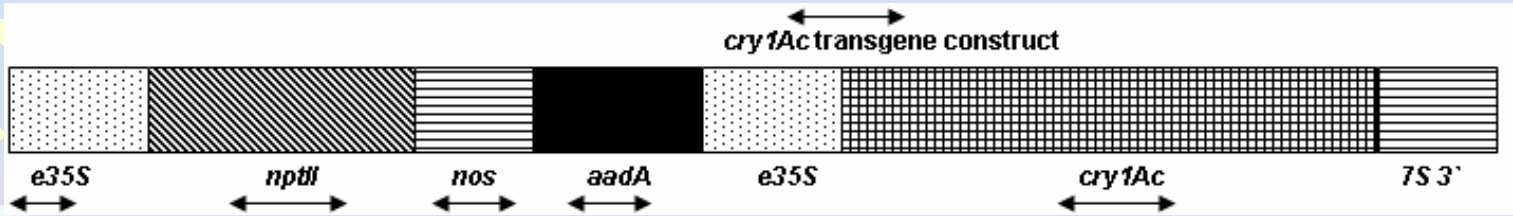
Source: **Randhawa G.J., M Singh, R Chhabra and R Sharma (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India. *Food Analytical Methods* DOI 10.1007/s12161-010-9126-8**

Construct-specific PCR

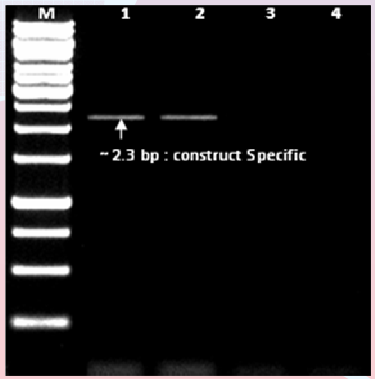
I.Bt-brinjal and Bt-rice *:* Juncture of 35S promoter and *cry1Ac* gene

II.GM Tomato: Juncture of 35S promoter and *avp1* gene in GM tomato line XAVP1D-2

III. GM Cotton: Juncture of 35S promoter and *cry1Ac* gene in Bt-cotton events *viz.* BG I, BG II, Event1 and GFM-cry1A

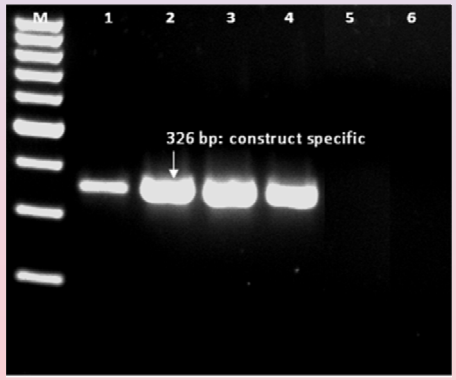


Linear Transgene Construct of MON 531



Construct-specific PCR for detection of a part of inserted gene construct in GM Tomato (*avp1* gene)

Lane1-2: GM tomato, Lane 3: Non-GM tomato, Lane 4: Water control, M: 1kb Ladder



Construct-specific PCR for detection of a part of inserted gene construct in GM cotton events *viz.* BGI, BGII, Event1 and GFM- cry1A

Lane 1: BGI, Lane 2: BGII, Lane 3: Event1, Lane 4: GFM-cry1A
Lane 5: Non-GM cotton, Lane 6: Water control, M: 100 bp ladder

Construct-specific PCR for detection of a part of inserted gene construct in GM rice (*cry1Ac* gene)

Lane 1: Water control, Lane 2: Non-GM rice, Lane 3-6: GM rice, M: 1kb ladder

Quantitative Detection

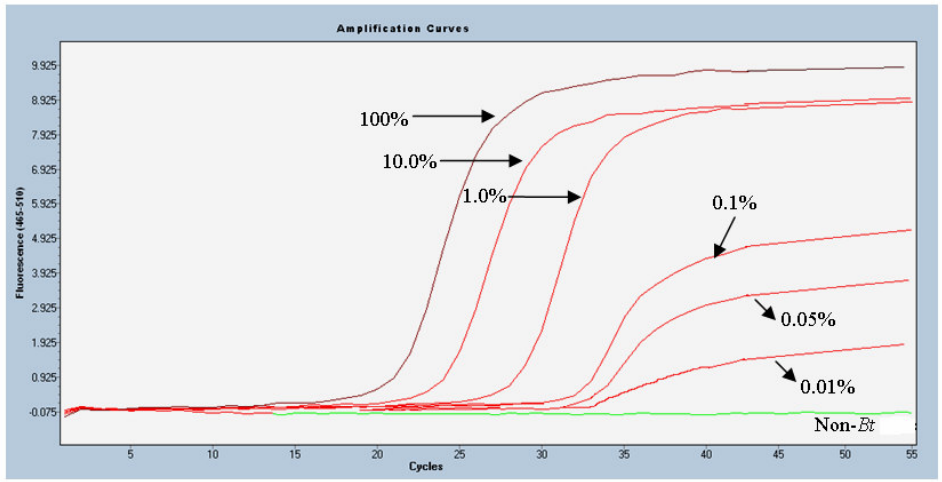
Real-time PCR assays on Light cycler®480 system

I.GM Tomato: GM tomato with *AVP1* gene

II. GM Cotton: Roundup Ready cotton Event MON 1445 with *EPSPS* gene

III. GM Potato: For insect resistance with *cry1Ab* gene

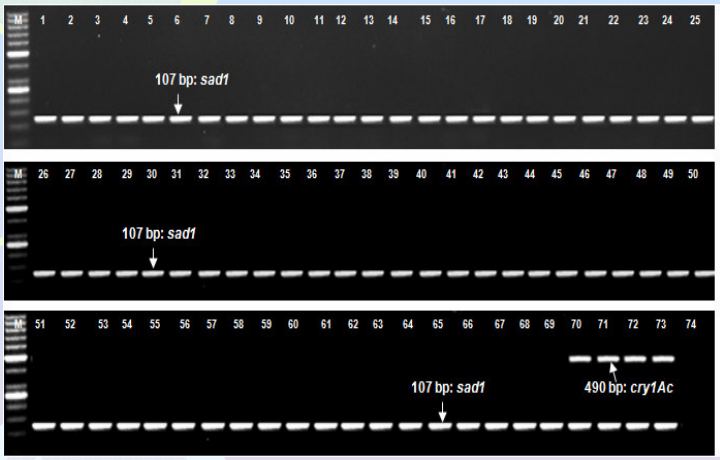
Sensitivity of Real-Time PCR assay for *cry1Ac* gene: up to 0.01%



The experimental mean values for GM content, *i.e.*, 100, 10, 1.0, 0.1, 0.05 and 0.01 ng/µl were found similar to the theoretical values indicating that the developed assays can detect as low as 0.01 ng of genomic DNA with *cry1Ac* gene.

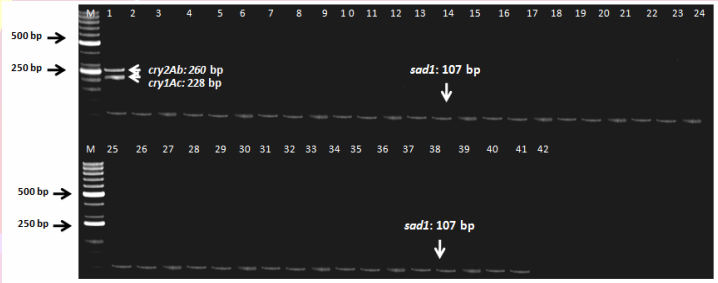
Test samples with 100, 10, 1.0, 0.1, 0.05 and 0.01% transgene content showed the amplification signals whereas no signal was detected in non-*Bt* sample

Study with *ex-situ* collections of Cotton Germplasm from National Gene Bank, NBPGR, New Delhi



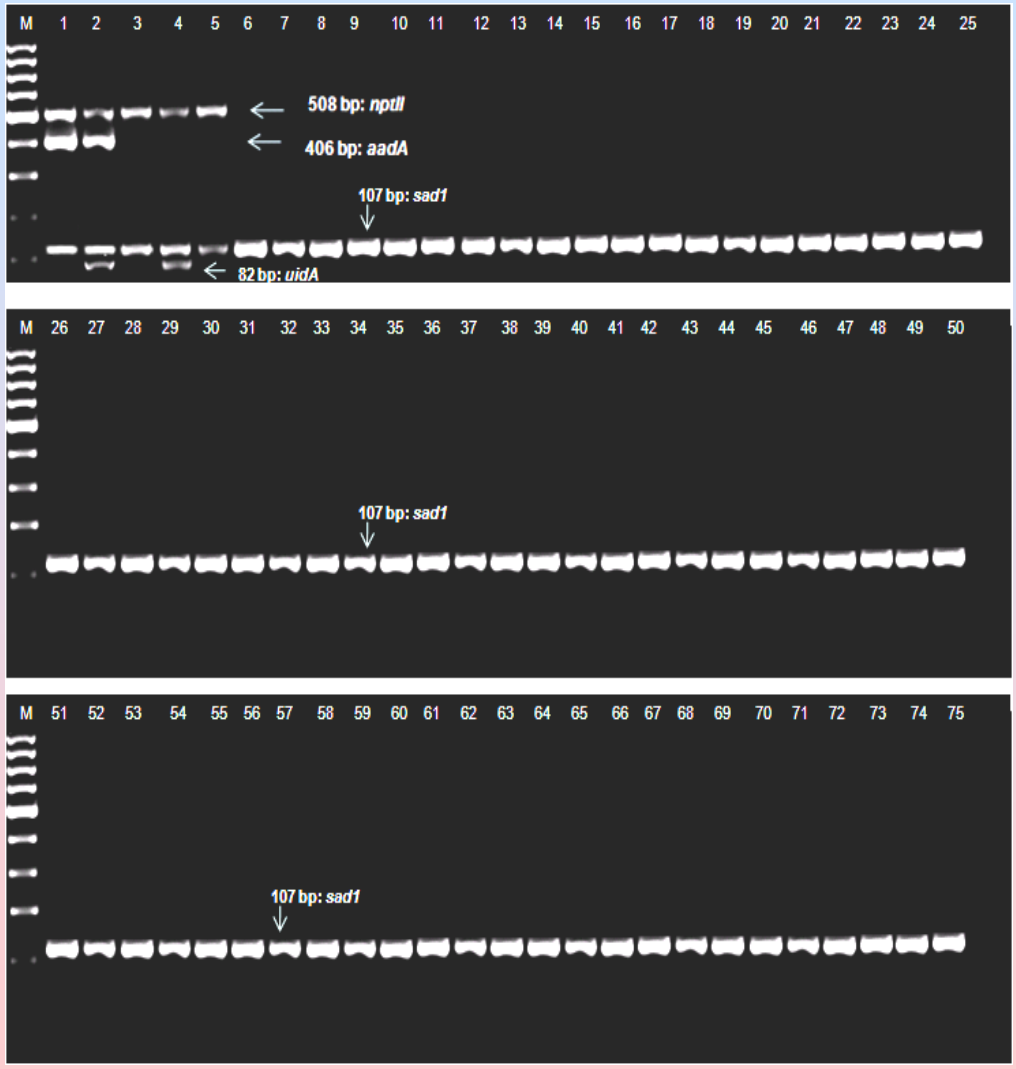
Multiplex PCR of 1-69 cotton accessions

Lane M: 50 bp, Lane 1-69: cotton accessions (Test samples) Lane 70: BGI; Lane 71: BGII; Lane 72: Event 1; Lane 73: GFM-cry1A; Lane 74: Water control



Multiplex PCR amplification of ex-situ cotton samples 1-40 with *cry1Ac, cry2Ab* and endogenous genes-specific primer pairs

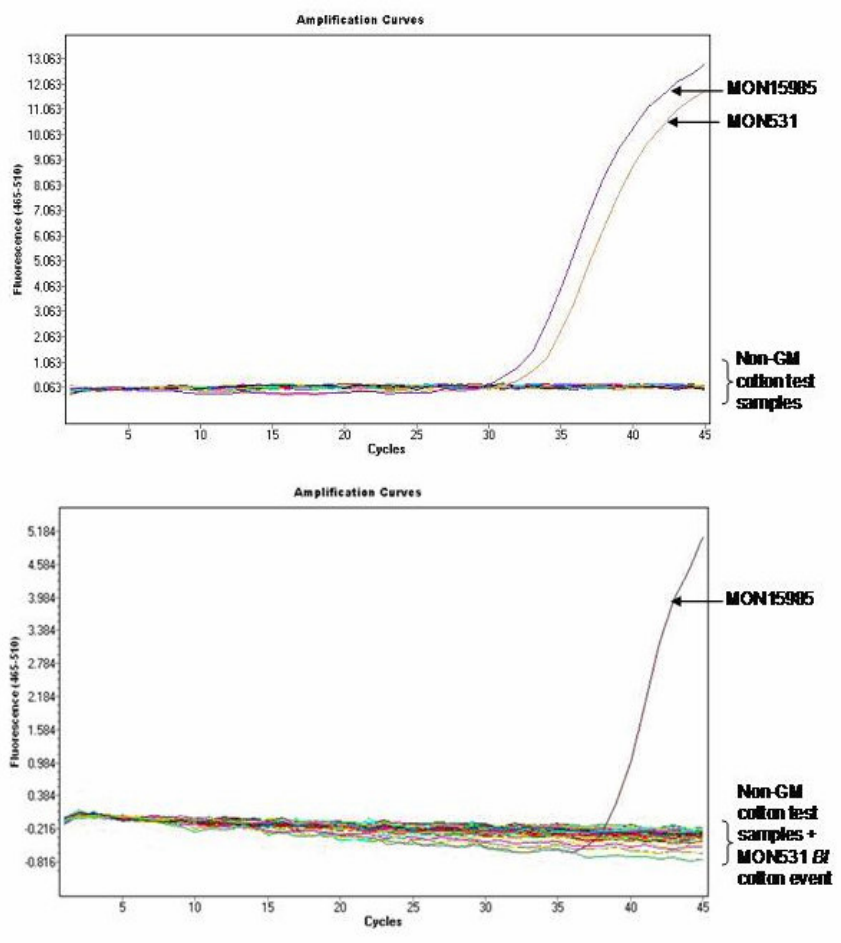
Lane M: 50 bp ladder, Lane 1: MON15985 Bt cotton event, Lane 2-41: *c*otton samples; Lane 42: water control



Multiplex PCR for screening of marker genes

Lane M: 100 bp ladder, Lane 1: BGI (***nptII, aadA***); Lane 2: BGII (***nptII, aadA, uidA)*** ; Lane 3: Event 1 (***nptII)***; Lane 4: GFM-cry1A (***nptII, uidA)*** , Lane 5: Dharwad event (***nptII)***, Lane 7-75: cotton accessions; Lane 6: Water control

123 accessions of *ex-situ* cotton collections were from 5 cotton growing states in India viz., Andhra Pradesh, Gujarat, Madhya Pradesh, Maharashtra and Tamil Nadu



Real-time PCR for monitoring of adventitious presence of transgenes in *ex situ* cotton collection

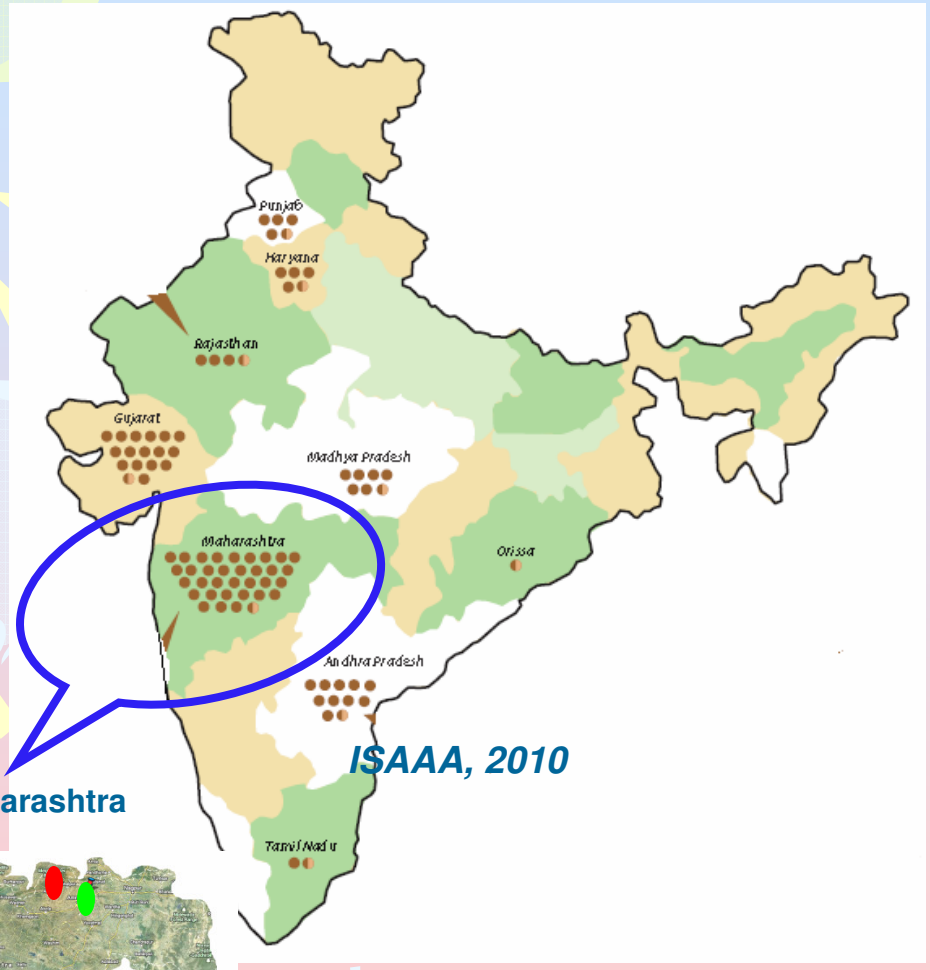
None of the cotton accessions has shown the adventitious presence of transgenes of commercialized Bt cotton events

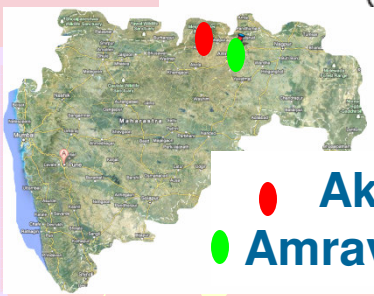
Study with 83 *ex-situ* collections of Brinjal Germplasm from National Gene Bank, NBPGR, New Delhi

Screening for adventitious presence of transgenes in 83 *ex-situ* Brinjal accessions was undertaken with *CaMV* 35S, *cry1Ab* gene and *nptII* marker gene-specific primers.

So far, no adventitious presence of transgenes was detected in brinjal accessions

Collection of 106 cotton accessions from Bt cotton growing regions in Maharashtra



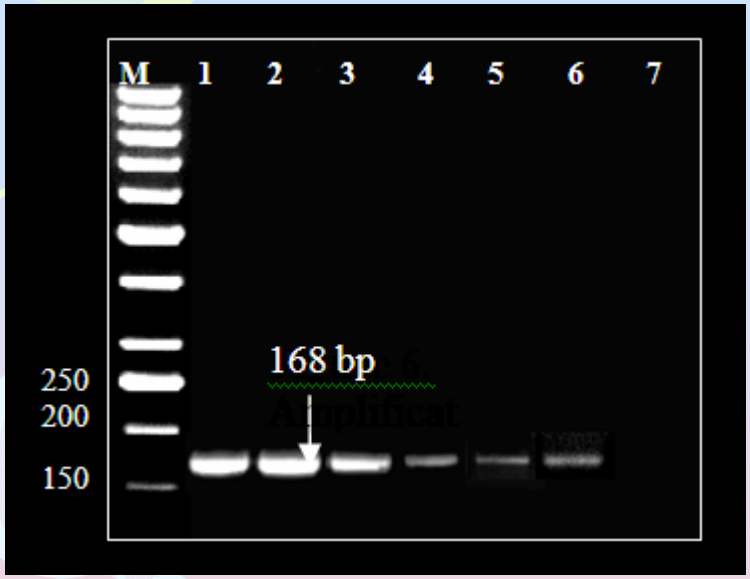


106 cotton samples were collected from from farmer fields of Akola (85) and Amravati (21) district of Maharashtra in consultation with Mr. Abdul Nizar, Office Incharge, NBPGR regional station, Akola (In Jan. 2012)

GM cotton (BGI & BGII)	Non-GM cotton	Total
24	82	106

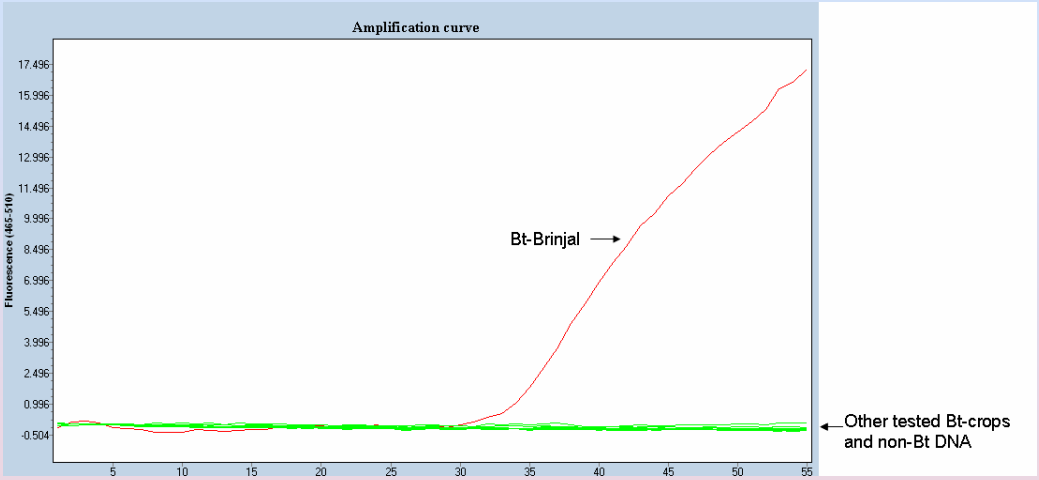
Preliminary screening for adventitious presence of transgene is under progress.

Event-specific Detection of Bt Brinjal Event EE-1



Sensitivity of Event-specific PCR assays

Lane M: 50bp ladder; Lane M: 1kb ladder, Lanes 1-6: Serial dilutions of *Bt* brinjal with 100, 10, 1.0, 0.1, 0.05, 0.01% of GM content, Lane 7: Non-*Bt* brinjal



Real-time event-specific PCR for Bt Brinjal Event EE-1 using specific probes

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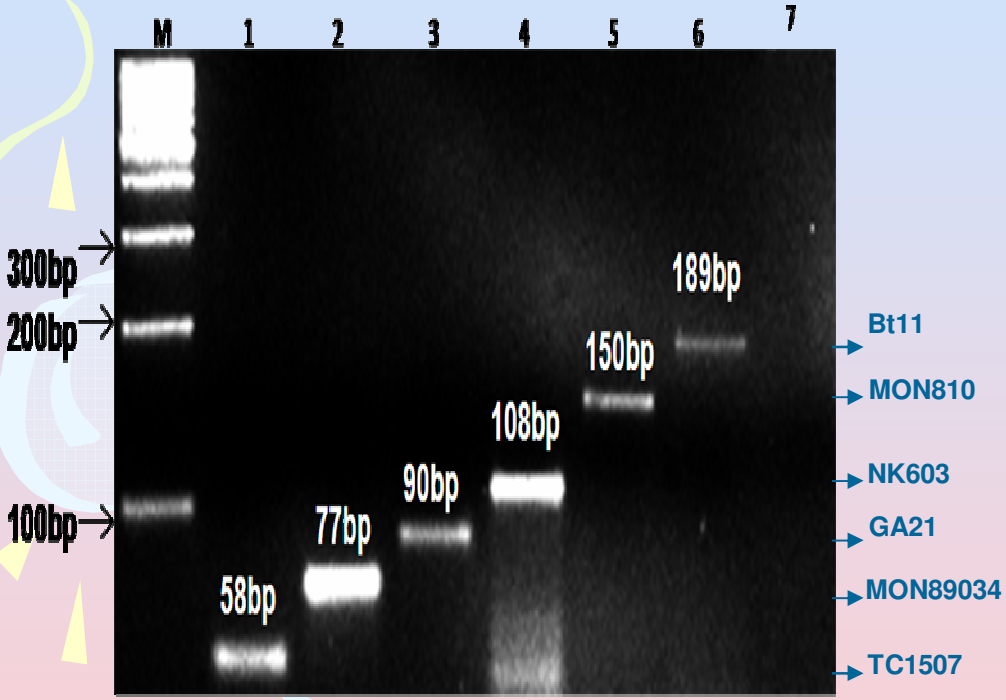
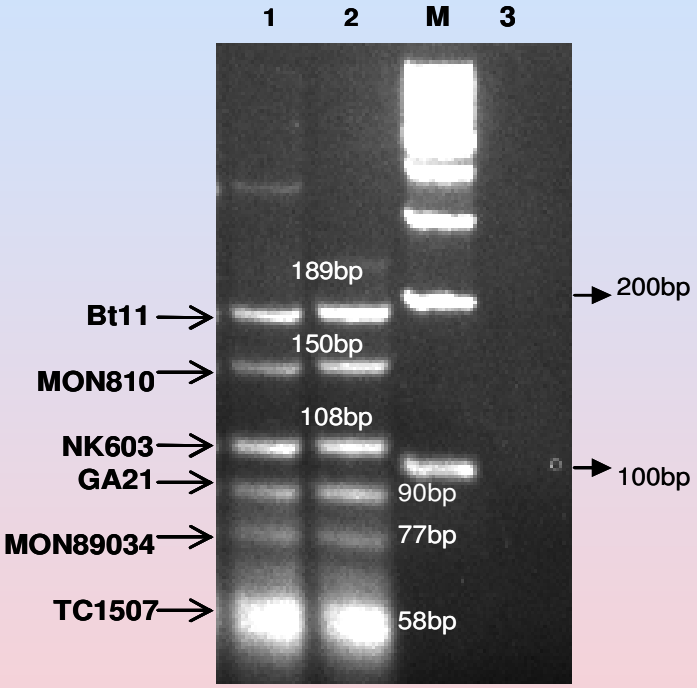
Source: Randhawa G.J, Sharma R and Singh M (2012) Qualitative and Event-Specific Real-Time PCR Detection Methods for *Bt* Brinjal Event EE-1. ***J. AOAC International*** (in press).

Event specific simplex and multiplex PCR for six GM Maize Events viz., Bt11, MON810, NK603, GA21, MON89034, TC1507

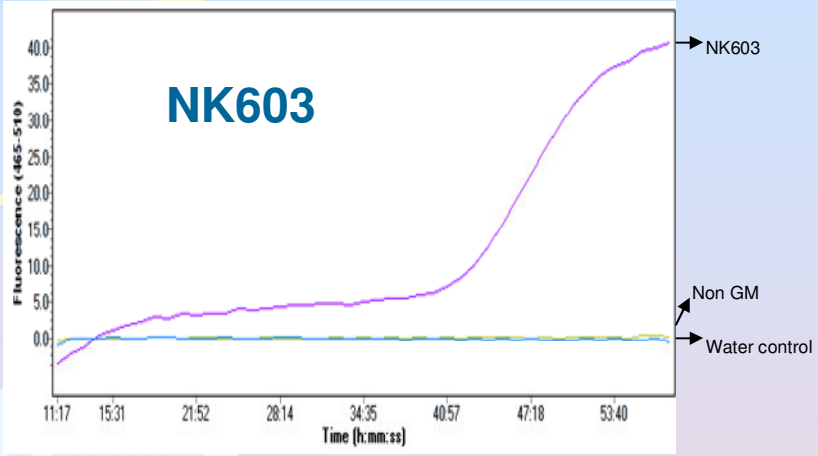


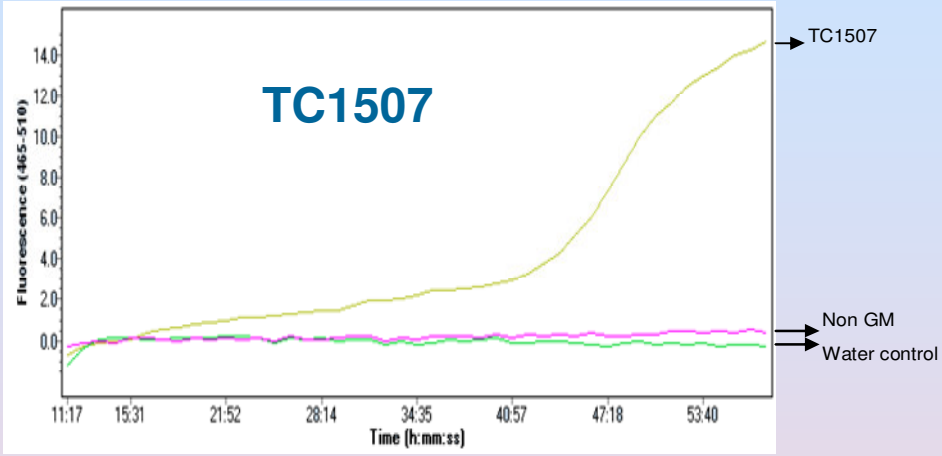
Simplex PCR for six GM Maize Events M- 100bp DNA marker; Lane1: TC1507, Lane 2: MON89034; Lane 3:GA21; Lane 4: NK603; Lane 5: MON810; Lane 6: Bt11; Lane-7: water control;



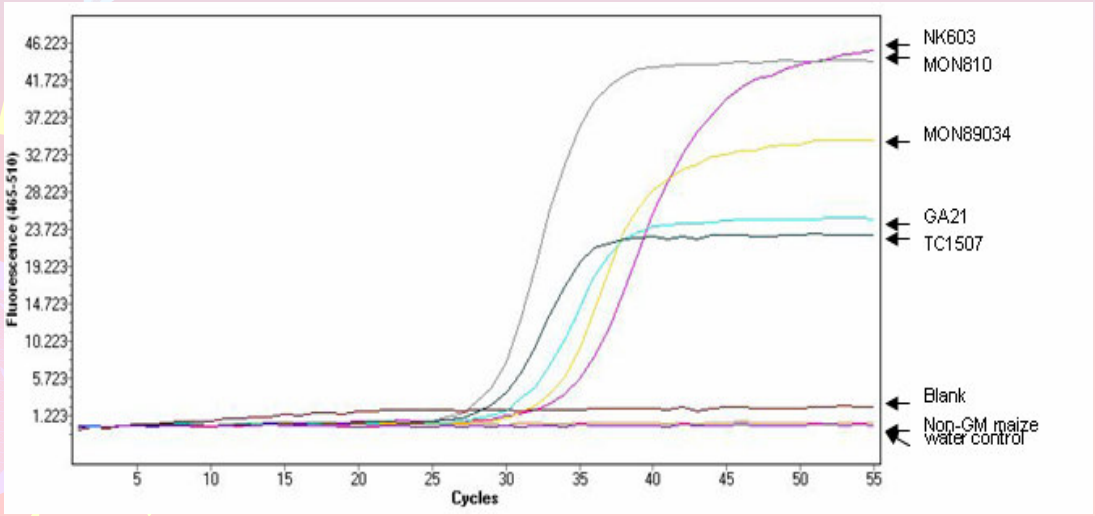
Hexaplex PCR for simultaneous detection of six GM Maize Events *viz.* Bt11, MON810, NK603, GA21, MON89034, TC1507; M- 100bp DNA marker; Lane-3: water control

TaqMan and SYBR-Green-based Real time PCR amplification of GM maize Events NK603, MON810, MON89034, GA21 and TC1507





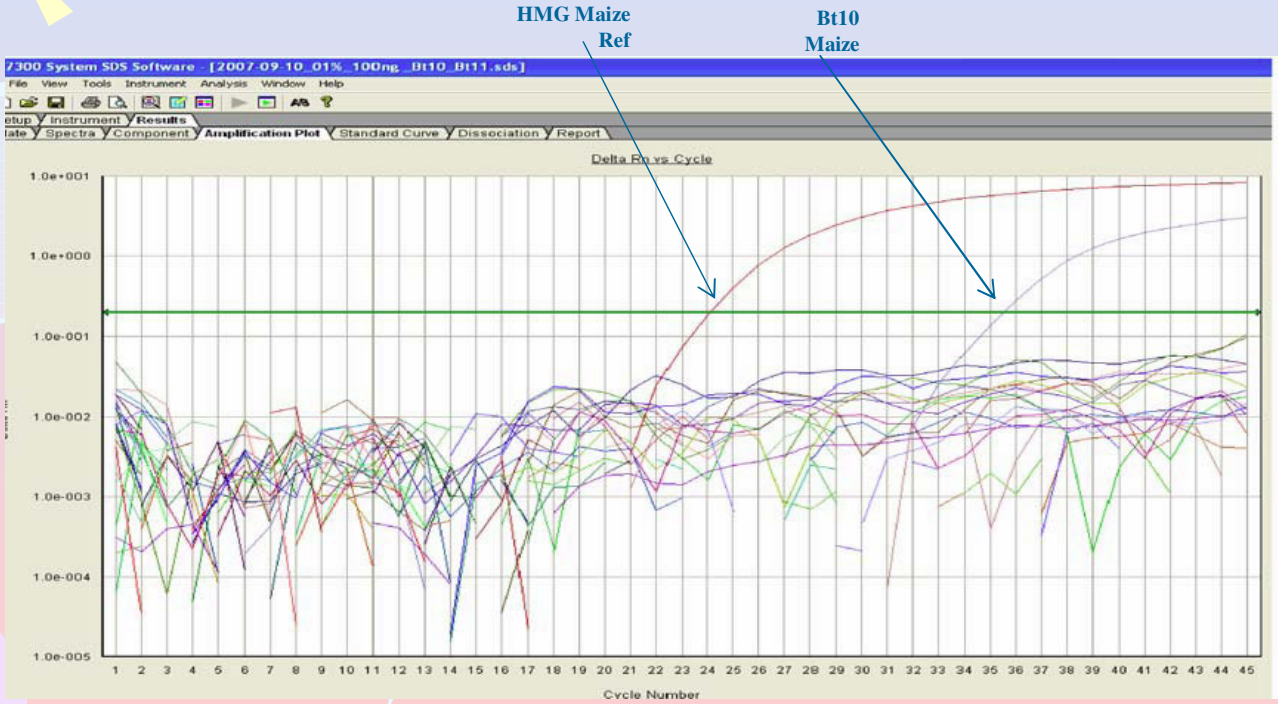
TaqMan based Real-time PCR



SYBR-Green based Real-time PCR

Validation of Multi Target Real-time PCR Plate

Ready to use pre–spotted plates containing, in lyophlized format, all primers and probes for the individual detection of 39 GM events and of the corresponding 7 plants species (maize, cotton, rice, oilseed rape, soybean, sugar beet and potato).



Amplification plot for the identification of 0.1% Bt10 maize. Curves above the threshold (green horizontal line) indicate positive reaction for maize reference gene and for event Bt10

Source: Querci M *et.al. (2009)* PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection, Food Anal. Methods 2:325–336.

Multi-target Plate showing GM Events used for Analysis

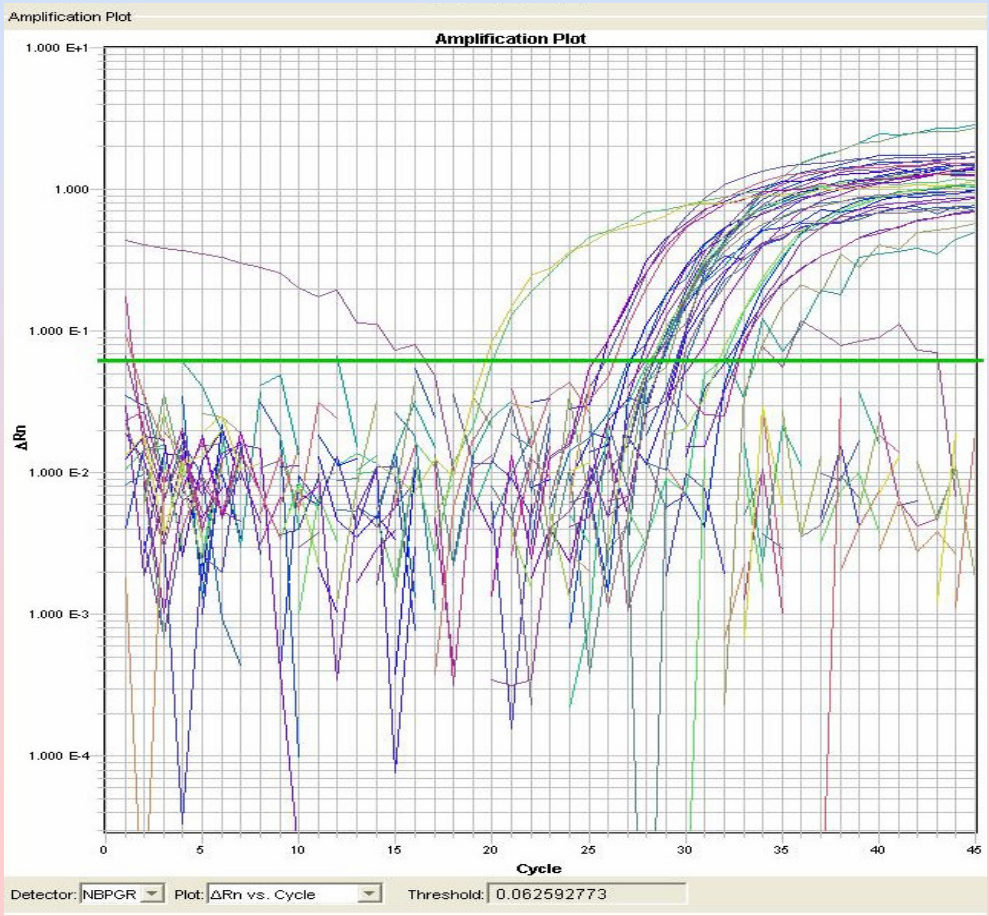
	1	2	3	4	5	6	7	8	9	10	11	12
A	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	1507 Maize	T25 Maize	59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize	LY038 Maize
B	3272 Maize	MON89034 Maize	98140 Maize	Bt176 Maize	SAH7 Cotton Ref	281-24-236 Cotton	3006-210-23 Cotton	LLCotton25 Cotton	MON 531 Cotton	MON1445 Cotton	MON15985 Cotton	MON88913 Cotton
C	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean	CruA Oilseed rape Ref	T45 Oilseed rape	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
D	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	PLD Rice Ref	LLRICE62 Rice	LLRice601 Rice	Bt63 Rice	GS Sugarbeet Ref	H7-1 Sugarbeet	UGPase Potato Ref	EH92-527-1 Potato
E	HMG Maize Ref	Bt11 Maize	NK603 Maize	GA21 Maize	MON863 Maize	1507 Maize	T25 Maize	59122 Maize	MON810 Maize	MIR604 Maize	MON88017 Maize	LY038 Maize
F	3272 Maize	MON89034 Maize	98140 Maize	Bt176 Maize	SAH7 Cotton Ref	281-24-236 Cotton	3006-210-23 Cotton	LLCotton25 Cotton	MON 531 Cotton	MON1445 Cotton	MON15985 Cotton	MON88913 Cotton
G	Lectin Soybean Ref	A2704-12 Soybean	40-3-2 Soybean	MON89788 Soybean	DP-356043 Soybean	DP-305423 Soybean	A5547-127 Soybean	CruA Oilseed rape Ref	T45 Oilseed rape	Ms8 Oilseed rape	Rf3 Oilseed rape	GT73 Oilseed rape
H	Rf1 Oilseed rape	Rf2 Oilseed rape	Ms1 Oilseed rape	Topas 19/2 Oilseed rape	PLD Rice Ref	LLRICE62 Rice	LLRice601 Rice	Bt63 Rice	GS Sugarbeet Ref	H7-1 Sugarbeet	UGPase Potato Ref	EH92-527-1 Potato

Positive Result

Imported GM Events of Maize, Cotton and Soybean used for Multi-Target Plate Analysis

S.No.	Events/Endogenous genes	Set 1	Set 2
Cotton			
1	281-24-236	√	√
2	3006-210-23	√	√
3	MON15985	√	√
4	MON531	√	√
5	MON88913	√	√
7	Sah 7 (endogenous)	√	√
Maize			
1	TC1507	√	√
2	Bt11	√	√
3	Bt176	√	√
4	MON810	√	√
5	GA21	√	√
6	MON89034	√	√
7	NK603	√	√
8	Hmg (endogenous)	√	√
Soybean			
1	40-3-2	√	√
2	Lectin (endogenous)	√	√
Rice			
1	PLD (endogenous)	√	√
Potato			
1	UGPase	√	√

Real Time PCR based Amplification Plot

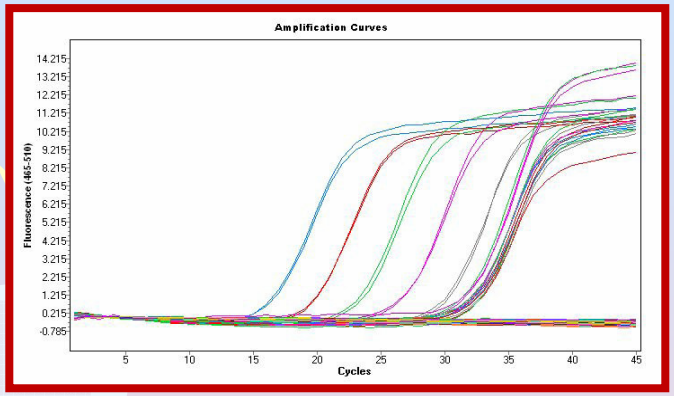


Participation in Five Real Time PCR based Comparative/Proficiency Testings Organized by Joint Research Centre, Italy, European Commission (4)

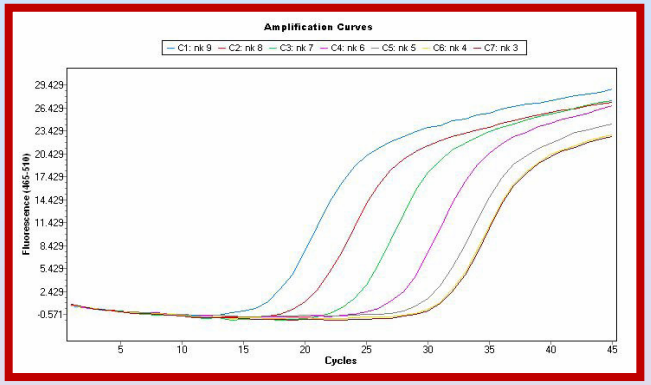
As per ISO/IEC 17043:2010 accreditation and Grain Inspection, Packers & Stockyards Administration

United States Department of Agriculture (GIPSA-USDA :1)

EU Comparative Testing I: For two unknown levels of NK603 GM maize event using Real Time-PCR in April, 2010



NK603 event gene of interest

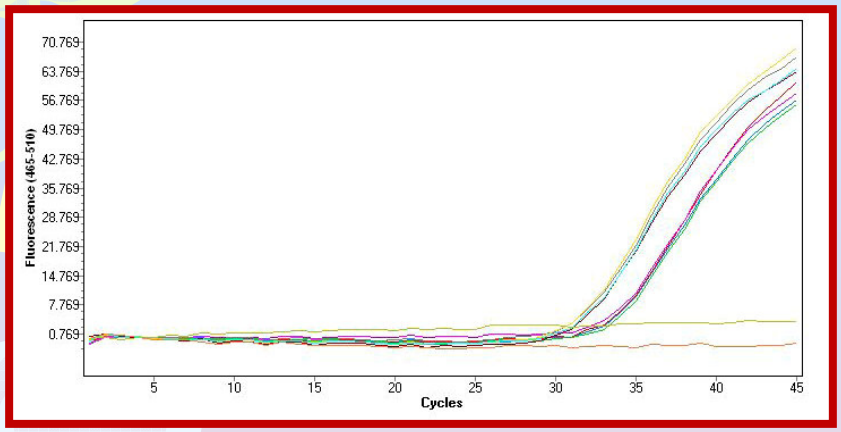


Adh1 endogenous gene

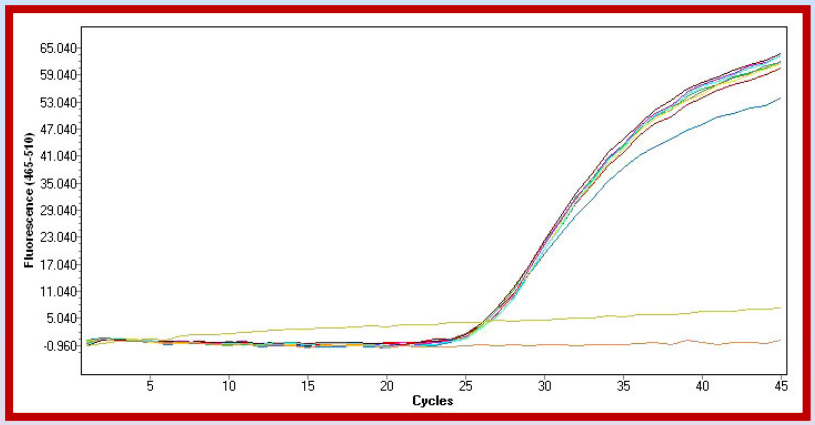
S. No	Sample	Sample details	Experimental Transgene %	Calculated transgene %
1	331	Relative Q	0.430	0.1
2	332	Relative Q	1.27	1.69
3	321	Absolute Q	0.409	0.1
4	322	Absolute Q	1.006	1.69

Values indicate the detected GM% in NK603 event of GM Maize for the two unknown levels of mixture with non GM crops quantified using RT-PCR

EU Comparative Testing II: For two unknown levels of MON810 GM maize event using Real Time-PCR in October, 2010



MON810 event

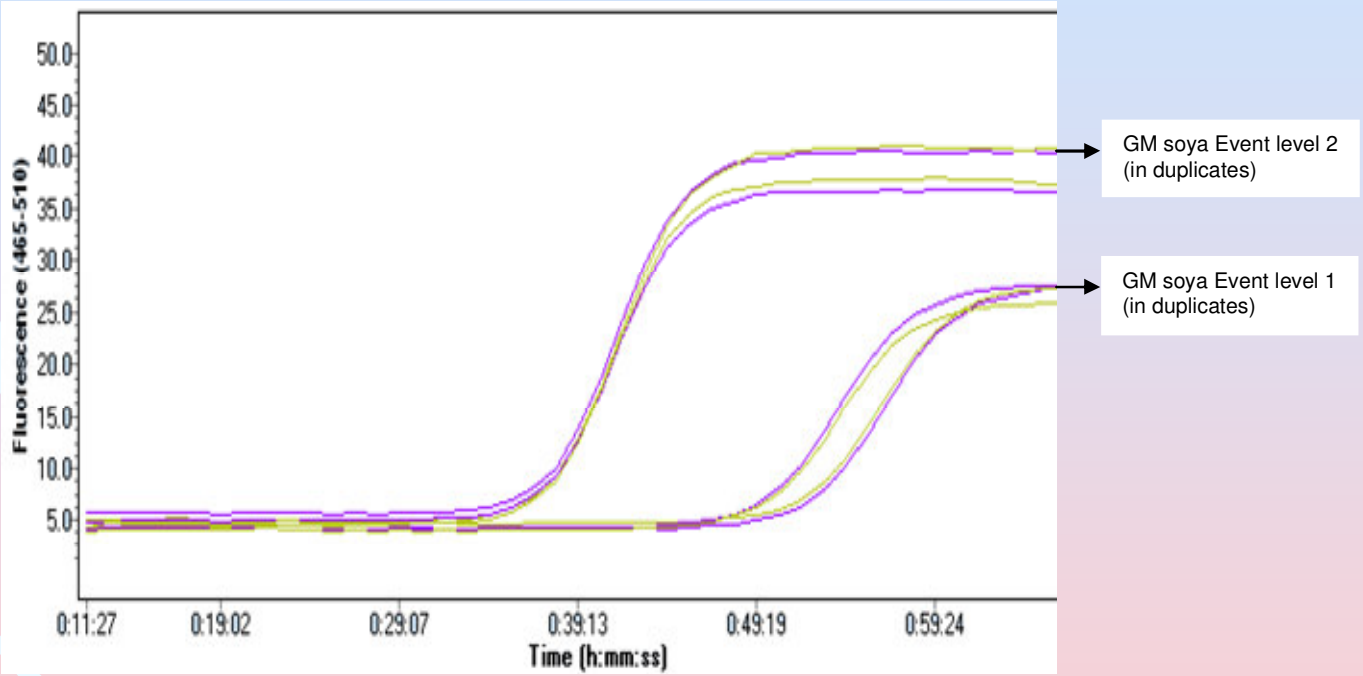


hmg endogenous reference gene

S. No	Sample	Sample details	Experimental Transgene %
1	006	Relative Q	0.52
2	050	Relative Q	2.50

Values indicate the detected GM% in MON810 event of GM Maize for the two unknown levels of mixture with non GM crops quantified using RT-PCR

EU Proficiency Testing III: April 2011
For two unknown levels of GM Soybean event 40-3-2



Real time PCR amplification plot for GM soya Event 40-3-2

GM content was estimated to be 1.56% and 6.04% in soybean powder level 1 and soybean powder level 2 respectively

EU Proficiency Testing IV : October 2011

For two unknown levels in blind samples of GM Maize

Qualitative screening was done for 10 GM maize events:

3272, Bt11, Bt176, 59122, GA21, MIR604, MON810, MON863, NK603, TC1507

Proficiency Testing (GIPSA-USDA): October 2011
For unknown levels of different GM maize events in 6 powdered samples on maize

Qualitative analysis was done for 12 GM maize events:

T25, CBH351, MON810, GA21, E176, Bt11, NK603, HerculexTM, Mon863, Herculex RW, Agrisure RW (MIR604), 3272, MON 88017, and MON 89034

"GM Chip Technology: Development and Applications"

- To plan for the strategy to develop the cost-effective microarray system for detection of GMOs in public-private partnership mode
- To review the developments of an innovative GM chip technology for molecular detection of GMOs

Participation

54 panelists/researchers participated

- Public sector: 38
- Private sector:16

On the Direction of Department of Biotechnology, Govt. Of India

Capacity building for addressing issues related to Biosafety & GM detection

Eight Orientation Courses on Biosafety Considerations for Evaluation of Transgenic Crops

(i) July 17-26, 2000

(ii) November 2-9, 2001

(iii) November 20-28, 2002

(iv) November 10-18, 2003

(v) November 22 to Dec- 1, 2004

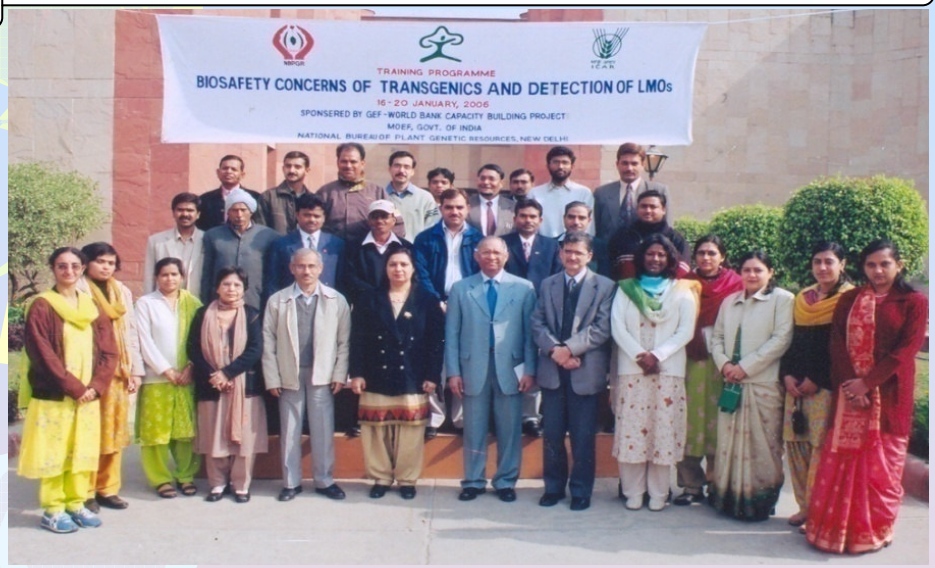
(vi) November 14 to 21, 2005

(vii) December 5 to 13, 2006

(viii) November 27 to Dec 5, 2007

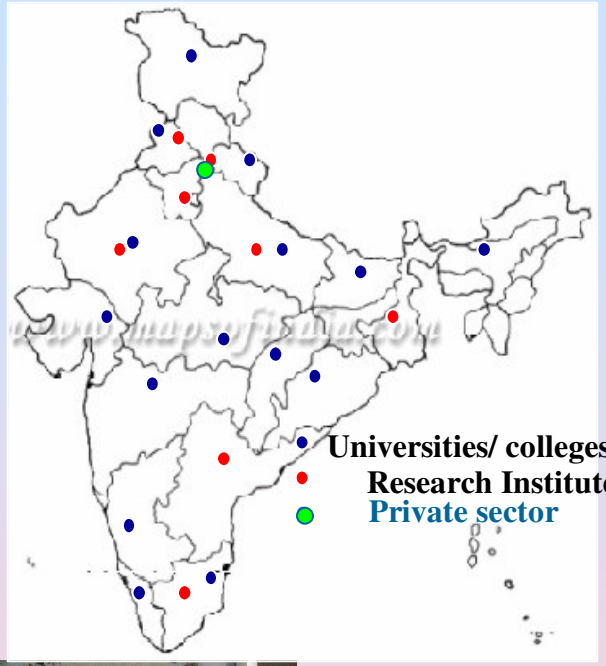
NBPGR

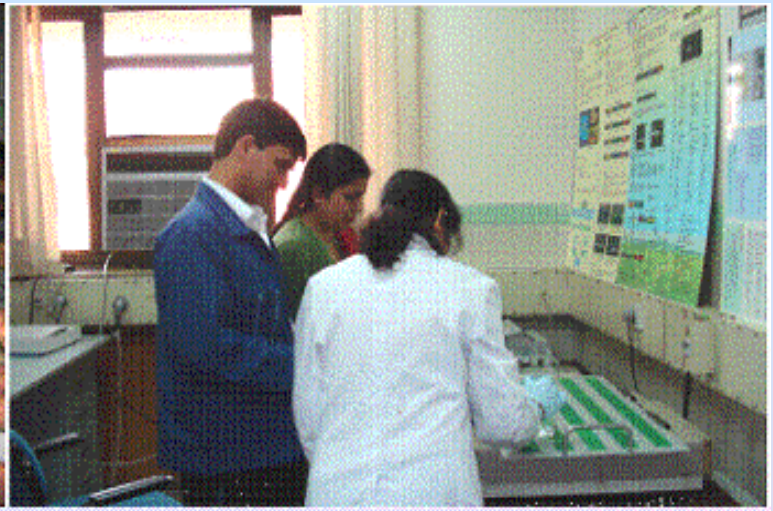
Biosafety Aspects of Transgenics and Detection of LMOs
14th to 21st March 2005
16th to 20th January 2006



Cartagena Protocol on Biosafety:Decisions to Diagnostics
23rd to 30th November 2006

Network of the Participants of the Training Programmes





More than 200 participants have been trained

NAIP sponsored training programme

Molecular Diagnostics for Risk Assessment and Management of Genetically Modified Crops

was organized from 8th to 21st November 2011 at NBPGR, New Delhi

Asia Sub-regional Training-of-Trainers Workshop on the Identification and Documentation of Living Modified Organisms

Jointly organized by NBPGR, New Delhi and ICGEB, New Delhi from 21 to 25 November 2011

33 Custom officials from 13 countries and four organizations participated in the training program

Technical Difficulties

1. Uniform global regulatory system for GM crops

   - ✓ Traceability
   - ✓ Labelling
   - ✓ Availability of certified reference materials

2. Need for detection and quantification of transgene after out crossing, zygosity testing of hybrids expressing transgenes

Further Collaborations

- To procure EU control samples/plasmids through MTA
- To develop a system of "validation/recommendation" of crop/species reference genes
- To establish on-line networking to allow exchange of information (e.g., research papers, announcements, training programmes, newsletter)

Publications

1. Randhawa G.J, Sharma R and Singh M (2012) Qualitative and Event-Specific Real-Time PCR Detection Methods for Bt Brinjal Event EE-1. *J. AOAC International* (in press).
2. Randhawa G.J. and Singh (2011) Multiplex, Construct-specific and Real Time PCR based Analytical methods for Bt rice with *cry1AC* gene. *J. AOAC International* 95 (1), 186-194.
3. Randhawa G.J., Chhabra R. and Singh M. (2011) PCR-based detection of GM tomato with *AVDI* gene employing seed sampling strategy. *Seed Sci. Technol* . 39, 112-124.
4. Randhawa G.J., Chhabra R. and Singh M. (2010) Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events. *J. Agric. Food Chem. 58* (18), 9875-9881.
5. Randhawa G.J., Singh M., Chhabra R. and Sharma R. (2010) Qualitative and Quantitative Molecular Testing Methodologies and Traceability Systems for Bt Crops Commercialised or Under Field Trials in India. *Food Anal. Methods* 3:295-303.
6. Randhawa G.J., Chhabra R. and Singh M. (2009) Multiplex PCR-based simultaneous amplification of selectable marker and reporter genes for screening of GM crops. *J. Agric. Food Chem.* 57, 5167-5172.
7. Randhawa G.J., Singh M. and Sharma R. (2009) Validation of *ST-LS1* as an endogenous reference gene for detection of *AmA1* and *cry1Ab* genes in GM potatoes using multiplex and real time PCR. *Am. J. Pot. Res.* 86:398-405.

Contd.....

7. Randhawa G.J. and Chhabra R. (2009) Import and Commercialization of Transgenic Crops: An Indian Perspective. *Asian Biotechnol. Develop. Rev.* 11(2) 115-130.

8. Randhawa G.J., Sharma R. and Singh M. (2009) Duplex, triplex and quadraplex PCR for molecular characterization of GM Potato with improved protein quality *Curr Sci.* 97 (1) 21-23.

9. Randhawa G.J., Sharma R. and Singh M. (2009) Multiplex PCR for detection of GM potato with *cry1Ab* gene. *Ind. J. Agric. Sci.* 79 (5): 368-71.

10. Randhawa G.J., Chhabra R. and Singh M. (2009) Molecular diagnosis of transgenic Tomato with *osmotin* gene using Multiplex PCR. *Curr Sci.* 96, No. 5: 689-694.

11. Randhawa G.J., Singh M., Chhabra R., Guleria S. and Sharma R. (2008) Molecular Characterization of Bt Cauliflower with Multiplex PCR and Validation of Endogenous Reference Gene in Brassicaceae Family. *Curr Sci.* (2008) 95, No.12 :1729-31.

12. Randhawa, G.J. and Firke, P.K. (2006) Detection of transgenes in GM soybean and maize using Polymerase Chain Reaction. *Ind. J. Biotech.* 5: 510-513.

Popular Articles

1. GJ Randhawa (2011) DNA-based Diagnostics for GM crops: Challenges ahead. *In*: **South Asian Biosafety Programme (SABP) Newsletter** 7 (11) pp 1-2.

2. GJ Randhawa (2010) DNA-based Diagnostics of Genetically Modified Crops: Regulation with Confidence. ***Biotech News***, 5 (5) 192-195.


Thank You!!