GM Crops and DNA based GM Diagnostics: Emerging Scenario and Challenges







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रा पा आ सं ब्यूरो
NBPGR

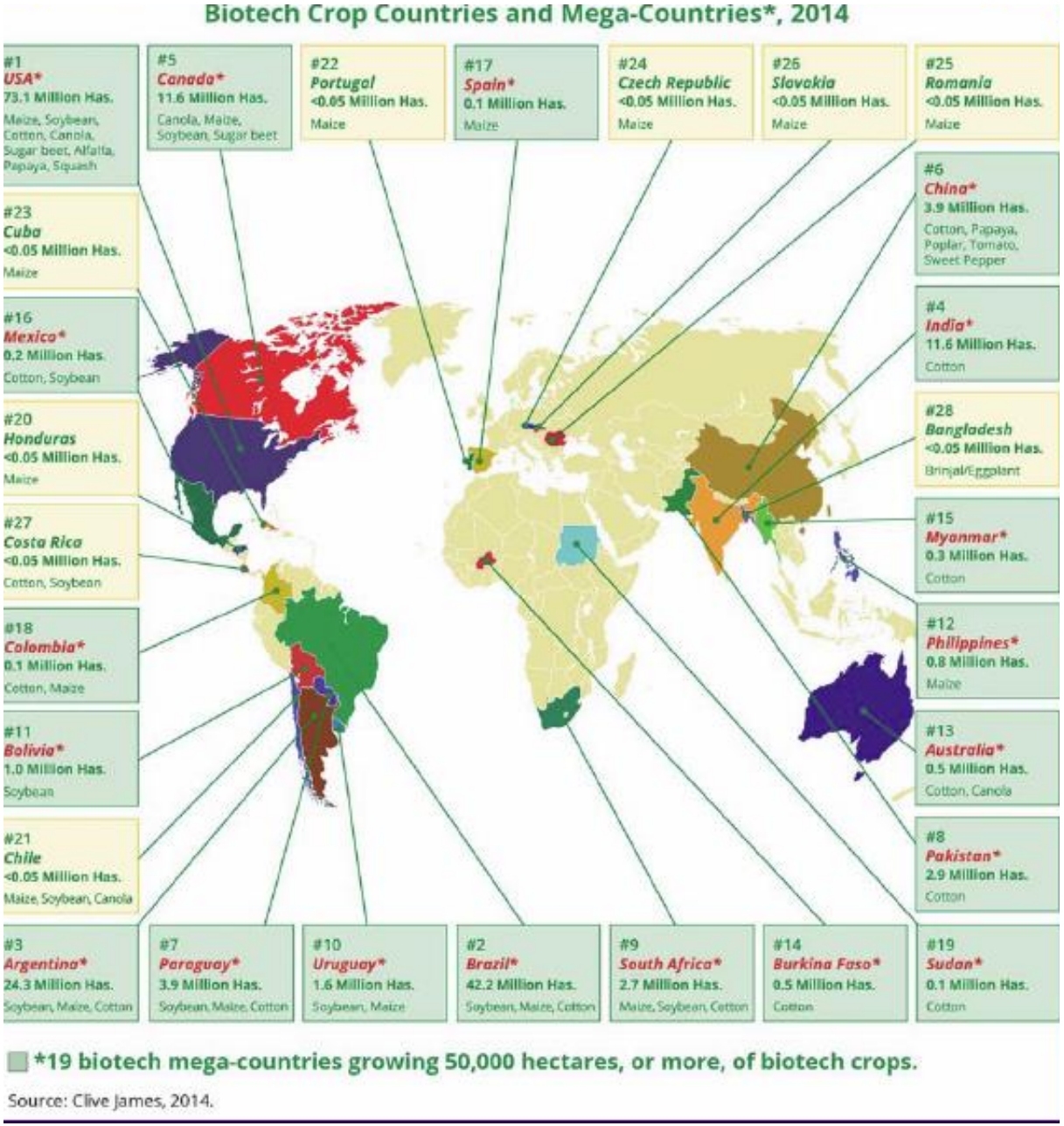
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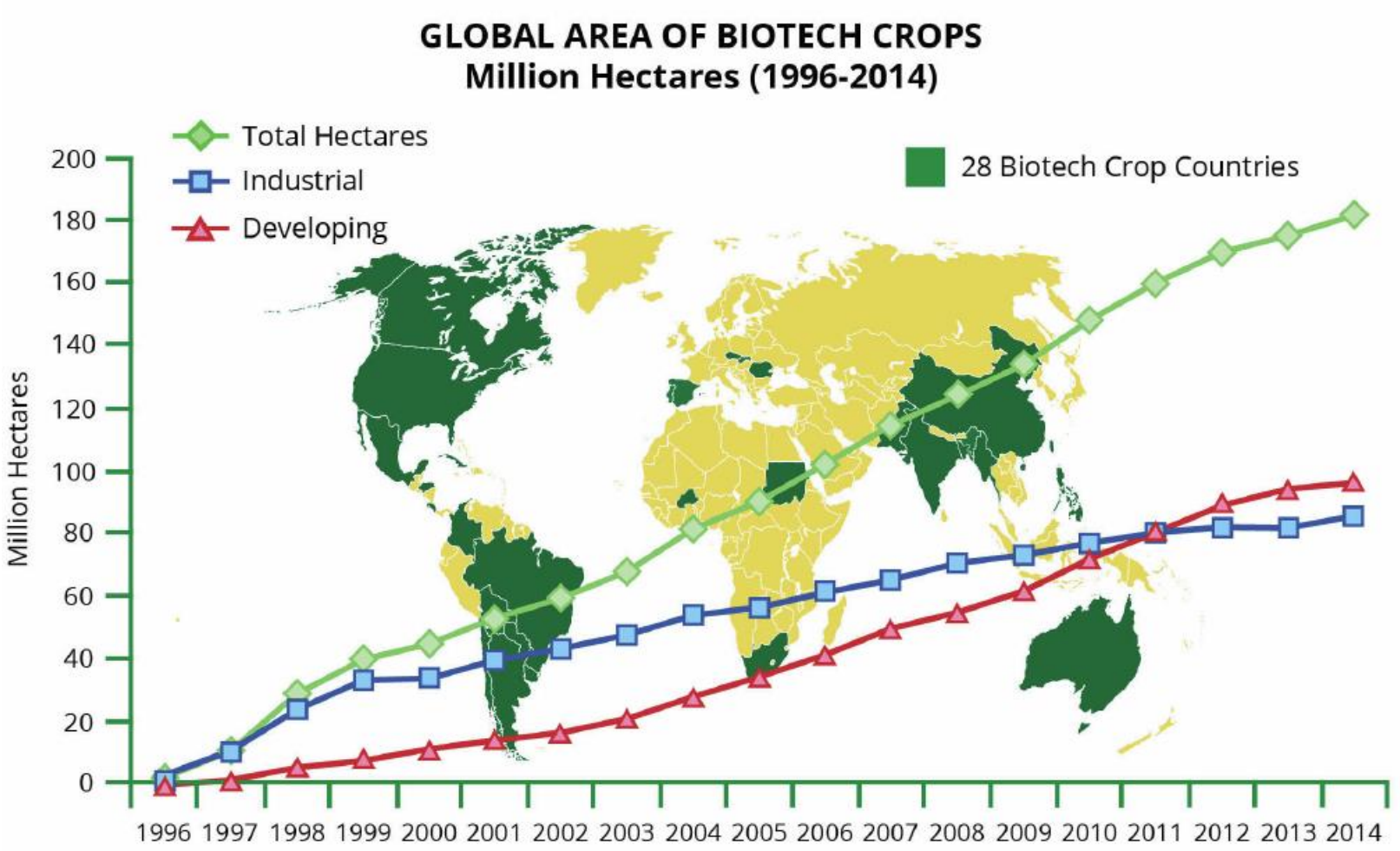


1. Global Status of GM Crops
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GM Crops growing Countries and Mega-countries, 2014



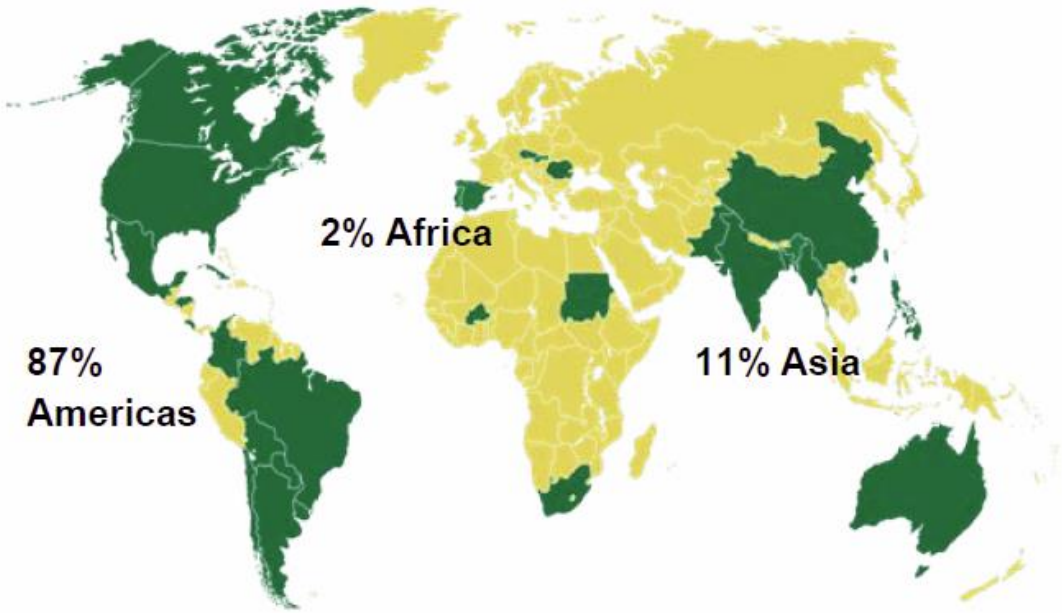
Global Area (million hectares) of GM Crops, (1996-2014)



A record 18 million farmers, in 28 countries, planted 181.5 million hectares (448 million acres) in 2014, a sustained increase of 3 to 4% or 6.3 million hectares (~16 million acres) over 2013.

Source: Clive James, 2014.

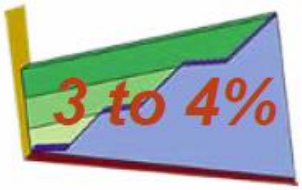
Global Area (million hectares) of GM Crops, 2014: By Country



28 countries which have adopted biotech crops

In 2014, global area of biotech crops was 181.5 million hectares, representing an increase of 3 to 4% over 2013, equivalent to 6.3 million hectares.

Increase over 2013



Source: Clive James, 2014.

Biotech Mega Countries

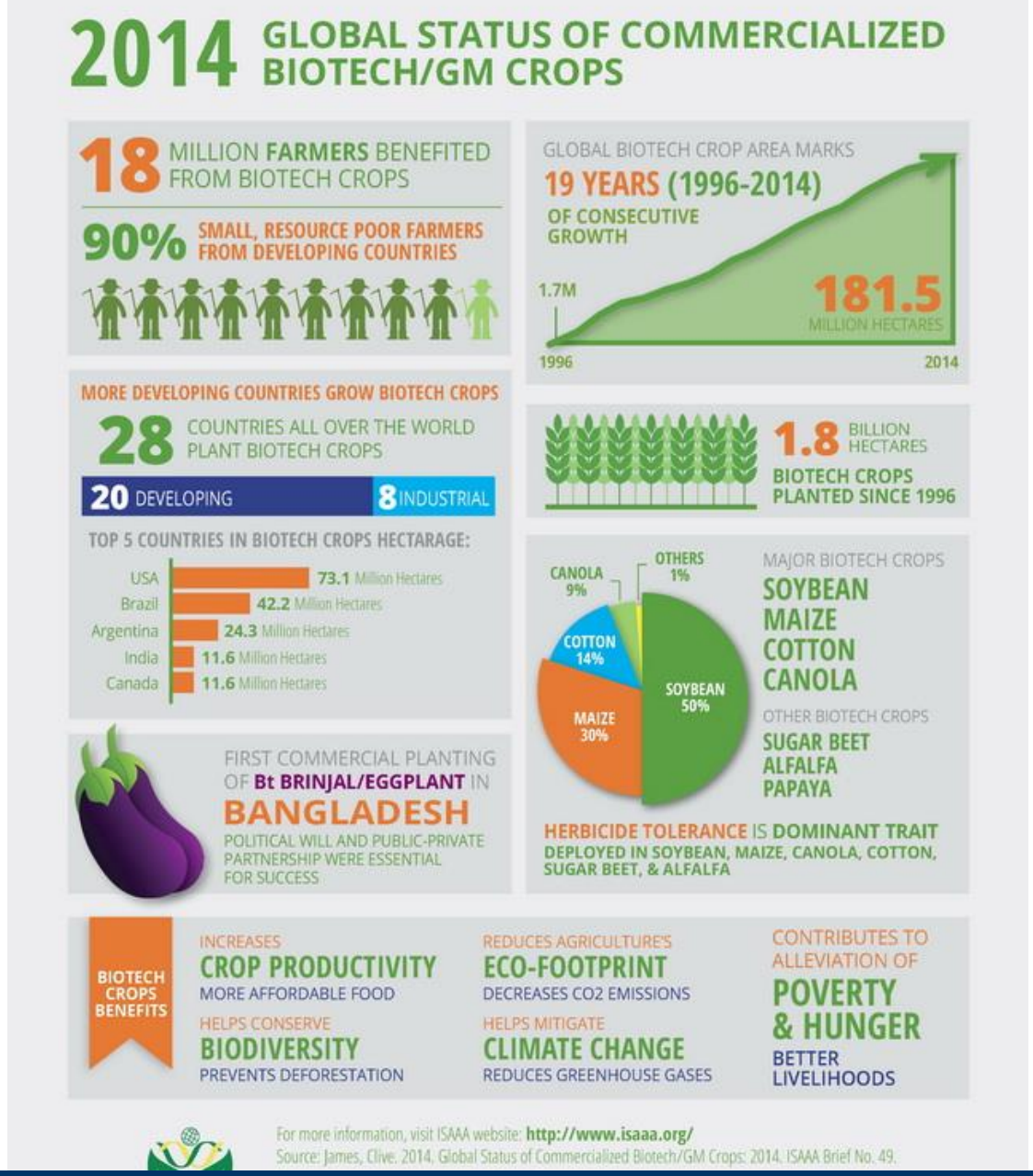
50,000 hectares (125,000 acres), or more

		Million Hectares
1.	USA	73.1
2.	*Brazil**	42.2
3.	*Argentina**	24.3
4.	*India**	11.6
5.	Canada	11.6
6.	*China**	3.9
7.	*Paraguay**	3.9
8.	*Pakistan**	2.9
9.	*South Africa**	2.7
10.	*Uruguay**	1.6
11.	*Bolivia**	1.0
12.	*Philippines**	0.8
13.	Australia	0.5
14.	*Burkina Faso**	0.5
15.	*Myanmar**	0.3
16.	*Mexico**	0.2
17.	Spain	0.1
18.	*Colombia**	0.1
19.	*Sudan**	0.1

Less than 50,000 hectares

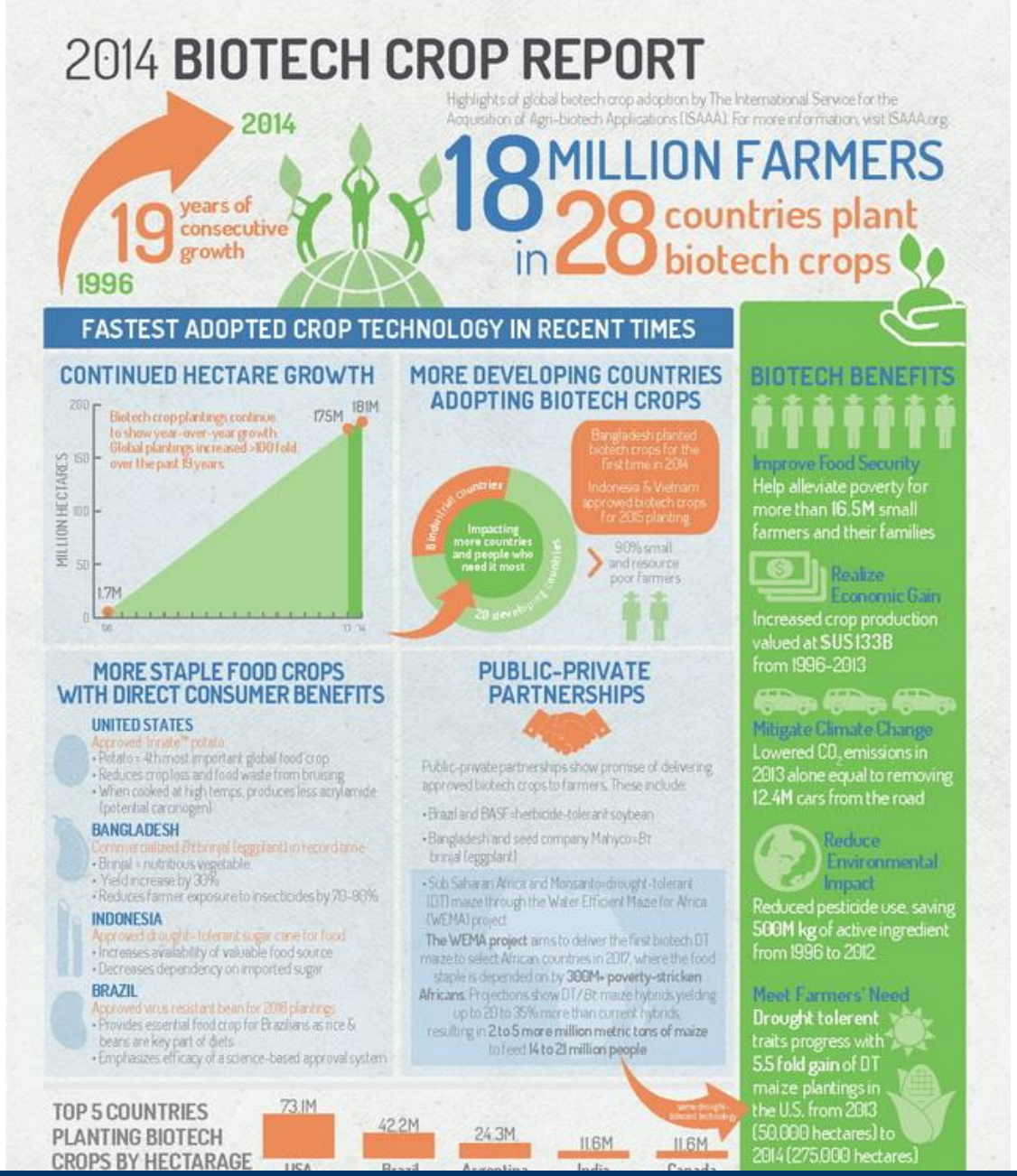
*Honduras**	Romania
*Chile**	Slovakia
Portugal	*Costa Rica**
*Cuba**	*Bangladesh**
Czech Republic	

** Developing countries*



An Overview of Global Adoption of GM Crops

Source: ISAAA



An Overview of Global Adoption of GM Crops

Source: ISAAA

GM Crops with Nutritionally Improved Traits

Trait	Crop (Trait Detail)	Reference
Protein and amino acids		
Protein quality and level	Bahiagrass (protein↑)	Luciani et al. (2005)
	Canola (amino acid composition)	Roesler et al. (1997)
	Maize (amino acid composition; protein↑)	Cromwell et al. (1967, 1969), O'Quinn et al. (2000), Yang et al. (2002), Young et al. (2004)
	Potato (amino acid composition; protein↑)	Yu and Ao (1997), Chakraborty et al. (2000), Li et al. (2001), Atanassov et al. (2004)
	Rice (protein↑; amino acid composition)	Katsube et al. (1999)
	Soybean (amino acid balance)	Dinkins et al. (2001), Rapp (2002)
	Sweet potato (protein↑)	Egnin and Prakash (1997)
Essential amino acids	Canola (Lys↑)	Falco et al. (1995)
	Lupin (Met↑)	White et al. (2001)
	Maize (Lys↑, Met↑)	Lai and Messing (2002), Agbios (2008)
	Potato (Met↑)	Zeh et al. (2001)
	Sorghum (Lys↑)	Zhao et al. (2003)
	Soybean (Lys↑, Trp↑)	Falco et al. (1995), Galili et al. (2002)
Oils and fatty acids		
	Canola (lauric acid↑, GLA↑; +ϖ-3 fatty acids; 8:0 and 10:0 fatty acids↑; lauric and myristic acids↑; oleic acid↑)	Dehesh et al. (1996), Del Vecchio (1996), Roesler et al. (1997), Froman and Ursin (2002, 2003), James et al. (2003), Agbios (2008)
	Cotton (oleic acid↑, oleic + stearic acids↑)	Chapman et al. (2001), Liu et al. (2002)
	Grass, legumes (↓trans-fatty acids)	O'Neill (2007)
	Linseed (+ϖ-3 and ϖ-6 fatty acids)	Abbadi et al. (2004)
	Maize (oil↑)	Young et al. (2004)
	Oil palm (oleic acid↑ or stearic acid↑, oleic acid↑, +palmitic acidY)	Jalani et al. (1997), Parveez (2003)
	Rice (α-linolenic acid↑)	Anai et al. (2003)
	Soybean (oleic acid↑, GLA↑)	Reddy and Thomas (1996), Kinney and Knowlton (1998)
	Safflower (GLA↑)	Arcadia Biosciences (2008)

Source: Martina (2008) Nutritionally approved agricultural crops. Plant Physiol. 147: 939-953

Trait	Crop (Trait Detail)	Reference
Carbohydrates		
Fructans	Chicory (fructan↑, fructan modification)	Smeekens (1997), Sprenger et al. (1997), Sévenier et al. (1998)
	Maize (fructan↑)	Caimi et al. (1996)
	Potato (fructan↑)	Hellwege et al. (1997)
	Sugar beet (fructan↑)	Smeekens (1997)
Fru, raffinose, stachyose	Soybean	Hartwig et al. (1997)
Inulin	Potato (inulin↑)	Hellwege et al. (2000)
Starch	Rice (amylase↑)	Schwall et al. (2000), Chiang et al. (2005)
Micronutrients and functional metabolites		
Vitamins and carotenoids	Canola (vitamin E↑)	Shintani and DellaPenna (1998)
	Maize (vitamin E↑, vitamin C↑)	Rocheford et al. (2002), Cahoon et al. (2003), Chen et al. (2003)
	Mustard (+β-carotene)	Shewmaker et al. (1999)
	Potato (β-carotene and lutein↑)	Ducreux et al. (2005)
	Rice (+β-carotene)	Ye et al. (2000)
	Strawberry (vitamin C↑)	Agius et al. (2003)
	Tomato (folate↑, phytoene and β-carotene↑, lycopene↑, provitamin A↑)	Rosati et al. (2000), Fraser et al. (2001), Mehta et al. (2002), Dı´az de la Garza et al. (2004), Enfissi et al. (2005), DellaPenna (2007)
Functional secondary metabolites	Apple (+stilbenes)	Szankowski et al. (2003)
	Alfalfa (+resveratrol)	Hipskind and Paiva (2000)
	Kiwi (+resveratrol)	Kobayashi et al. (2000)
	Maize (flavonoids↑)	Yu et al. (2000)
	Potato (anthocyanin and alkaloid glycoside↓, solanin↓)	Lukaszewicz et al. (2004)
	Rice (flavonoids↑, +resveratrol)	Stark-Lorenzen et al. (1997), Shin et al. (2006)
	Soybean (flavonoids↑)	Yu et al. (2003)
	Tomato (+resveratrol, chlorogenic acid↑, flavonoids↑, stilbene↑)	Rosati et al. (2000), Muir et al. (2001), Niggeweg et al. (2004), Giovinazzo et al. (2005)
	Wheat (caffeic and ferulic acids↑, +resveratrol)	United Press International (2002)
Mineral availabilities	Alfalfa (phytase↑)	Austin-Phillips et al. (1999)
	Lettuce (iron↑)	Goto et al. (2000)
	Rice (iron↑)	Lucca et al. (2002)
	Maize (phytase↑, ferritin↑)	Drakakaki et al. (2005)
	Soybean (phytase↑)	Denbow et al. (1998)
	Wheat (phytase↑)	Brinch-Pedersen et al. (2000)

GM Crops with Enhanced for Mineral and Vitamin Content

Nutrient	Species	Genes used	Total increase (fold increase over wild type)	References
Vitamin A	Maize *(Zea mays)*	*PacrtB and PacrtI*	33.6 µg/g DW (34)	Aluru et al. (2008)
	Maize *(Z. mays)*	*Zmpsy1, PacrtI, PcrtW, Gllycb*	146.7 µg/g DW (133)	Zhu et al. (2008)
	Maize *(Z. mays)*	*Zmpsy1, PacrtI*	163.2 µg/g DW (112)	Naqvi et al. (2009b)
	Wheat *(Triticum aestivum)*	*Zmpsy1, PacrtI*	4.96 µg/g DW (10.8)	Cong et al. (2009)
	Potato *(Solanum tuberosum)*	*EuCrtB, EuCrtI, EuCrtY*	114 µg/g DW (20)	Diretto et al. (2007)
	Potato *(S. tuberosum)*	*BoOr*	28.22 µg/g DW (6)	Lopez et al. (2008)
	Potato *(S. tuberosum)*	*AtZEP*	60.8 µg/g DW (5.7)	Romer et al. (2002)
	Potato *(S. tuberosum)*	*PacrtB*	35.5 µg/g DW (6.3)	Ducreux et al. (2005)
	Cassava *(Manihot esculenta)*	*PacrtB*	21.84 µg/g DW (33.6)	Welsch et al. (2010)
	Rice *(Oryza sativa)*	*Nppsy1, EucrtI*	1.6 µg/g	Ye et al. (2000)
	Rice *(O. sativa)*	*Zmppsy1, EucrtI*	37 µg/g (23)	Paine et al. (2005)
Vitamin C	Corn *(Z. mays)*	*Osdhar*	110 µg/g DW (6)	Naqvi et al. (2009b)
	Tomato *(Solanum lycopersicum)*	*Acggp*	46–111 mg/100 g FW (3–6)	Bulley et al. (2011)
	Potato *(S. tuberosum)*	*StVTC2A*	1.65 mg/g FW (3)	Bulley et al. (2011)
Folic acid	Rice *(O. sativa)*	*Atgtpchi, Atadcs*	38.3 nmol/g (100)	Storozhenko et al. (2007)
	Tomato *(S. lycopersicum)*	*Atgch, Atadcs1*	25 nmol/g (25)	Storozhenko et al. (2007)
Iron (Fe)	Rice *(O. sativa)*	*Osnas2*	19 µg/g DW in polished seeds (4.2)	Johnson et al. (2011)
	Rice *(O. sativa)*	*Gm ferritin, Af phytase, Osnas1*	7 µg/g DW in polished seeds (4–6.3)	Wirth et al. (2009)
	Rice *(O. sativa)*	*Activation tagging of*	32 in lg/g DW in dehusked (2.9)	Lee et al. (2009)
	Corn *(Z. mays)*	*Osnas3*	30 µg/g DW in whole seed (2)	Drakakaki et al. (2005)
	Cassava *(M. esculenta)*	*Gm ferritin and Af phytase*	40 µg/g DW in tuber	Sayre et al. (2011)
Zinc (Zn)	Rice *(O. sativa)*	*Crfea1*	40–45 lg/g DW in polished seeds	Lee et al. (2011)
	Rice *(O. sativa)*	*Activation tagging of*	(2.9)	Johnson et al. (2011)
	Rice *(O. sativa)*	*Osnas2*	52–76 µlg/g DW in polished seeds	Wirth et al. (2009)
		Osnas2	(2.2)	
	Cassava *(M. esculenta)*	*Gm ferritin, Af phytase, Osnas1*	35 µg/g DW in polished seeds (1.6)	Sayre et al. (2011)
	Cassava *(M. esculenta)*	*Atzat1*	Tuber (4)	Sayre et al. (2011)
Selenium (Se)	Indian Mustard *(Brassica juncea)*	*Atzip*	Tuber (2–10)	Pilion-Smits et al. (1999)
	Indian mustard *(B. juncea)*			LeDuc et al. (2004)
Calcium (Ca)	Carrot *(Daucus carota)*	*Ataps1*	(2–3)	Park et al. (2004)
	Lettuce *(Lactuca sativa)*	*Absmt1*	(2–4)	Park et al. (2009)
	Potato *(S. tuberosum)*	*scax1*	3.9 mg/g DW (1.6)	Park et al. (2005)
		scax1	18.9 mg/g DW (1.3)	Kim et al. (2006)

Source: Pérez-Massot et al. (2013) The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes Nutr. 8(1): 29–41.

Status of GM crops in India

- **Commercialized**
  (Area under *Bt* cotton: 11.6 mha)



- **Under development** by public (including ICAR)& private sector

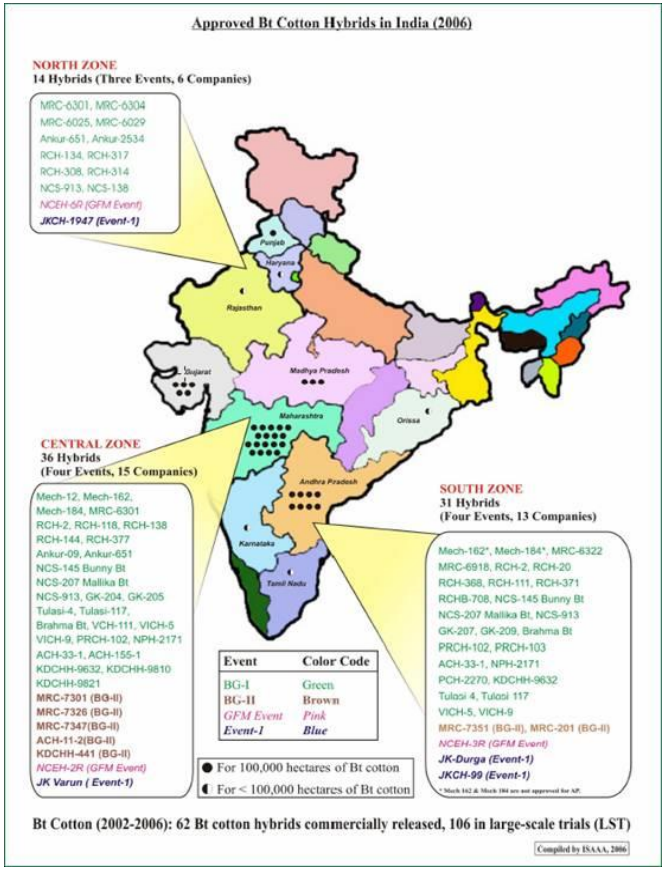


- **Imported** for research purposes



- Under different stages of **field trials**



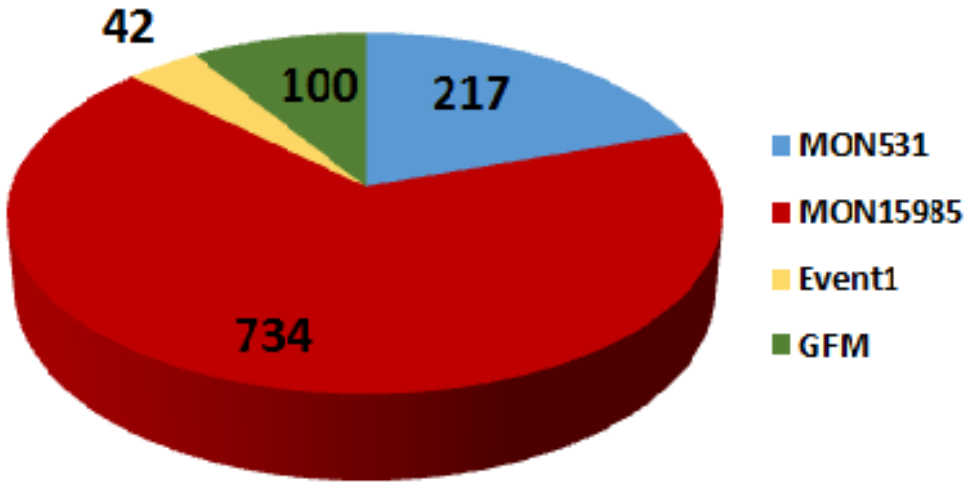


GM Events Commercialized in India

Area under commercial cultivation of GM crops: 11.6 mha

Event	Developer	Transgenes	Year of approval
MON531 (Bollgard® I)	Mahyco/Monsanto	*cry1Ac*	2002
MON15985 (Bollgard® II)	Mahyco/Monsanto	*cry1Ac, cry2Ab*	2006
GFM-*cry1A*	Nath Seeds	Fused *cry1Ab-Ac*	2006
Event 1	J. K. Agri-Genetics Limited	*cry1Ac*	2006

Approved *Bt* Cotton Hybrids



Source: James, 2014

GM Events Approved for Field Trials in India (2014-15)

THE HINDU
BusinessLine

Maharashtra approves field trials for four GM crops

OUR BUREAUS



Brinjal, rice, chickpea and cotton strains to be tested on university farms

New Delhi/Mumbai, January 30:

The Maharashtra government has decided to allow agricultural bodies and companie genetically-modified (GM) crops. According to a source in the know, no-objection cer for trials of brinjal, rice, chickpea (chana) and cotton on Thursday.

2015: No-objection certificates given for conducting field trials of GM brinjal, rice, chickpea and cotton in Maharashtra by the Maharashtra Govt.

2014: GEAC approved the field trials of transgenic mustard, *Bt* chickpea, GM rice for nutritional enhancement and *Bt* brinjal

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NEWS » NATIONAL

NEW DELHI, July 18, 2014

Updated: July 18, 2014 19:34 IST

GEAC clears field trials for GM crops

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MEENA MENON

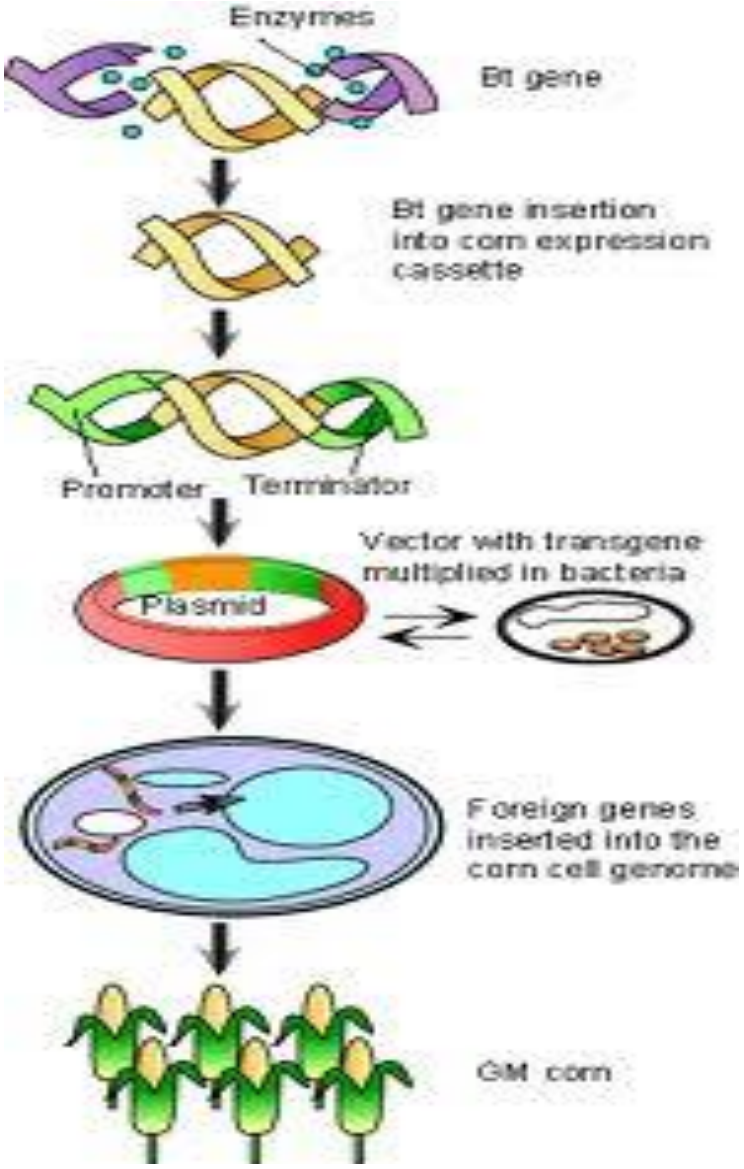
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TOPICS
politics
government

The Genetic Engineering Approval Committee (GEAC) on Friday gave the green signal for field trials of genetically modified (GM) rice, mustard, cotton, chickpea and brinjal at its meeting in Delhi.

Development of GM Crops



Source: http://files.gmosafety.webnode.com/200000030-6c0a67bad/genetransferring.gif

Purpose of Detection Methods

To assure purity and segregation of seeds and products thereof

To be able to trace genetic modification in breeding



To assure compliance with legislation

To be able to retrieve specific transgenic planting material in case of unauthorized material is being grown



To solve legal/IPR issues if they arise

Testing of GM Crops

Detection

To determine whether a sample contains any transgene



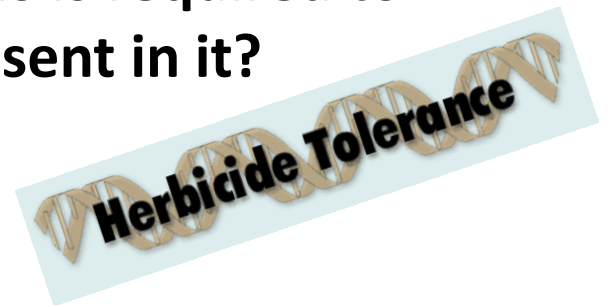
OR



Identification

If the results are positive, further analysis is required to find which specific transgene is present in it?





Quantification

If the specific transgene has been identified, the next step is to assess compliance with threshold level (e.g.1%)

MOLECULAR DIAGNOSIS OF GM MATERIAL

PCR with endogenous reference gene

-ve → DNA is contaminated with PCR inhibitors

+ve

Initial screening for control elements viz., 35S promoter, terminator, marker genes etc., by simplex PCR

-ve → Sample is non-GM

+ve



Sample is GM

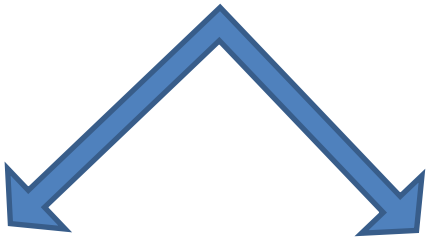
Identification of specific transgene by simplex PCR /multiplex PCR

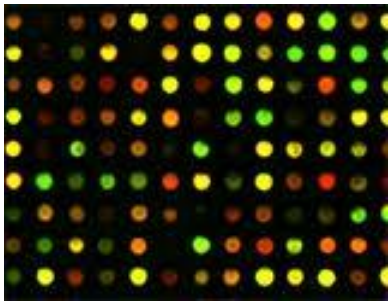
Construct-/event-specific PCR for more specificity of the particular event

Quantification using Real-time PCR to check the % GM content and copy number of the transgene and to test the contamination upto 0.01% as per the instructions of Hon'ble Supreme Court

Methods of GMO detection



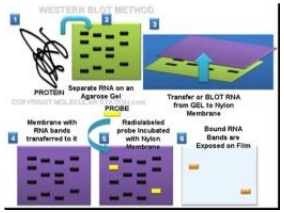




Low technological methods
Protein-based Methods

- Herbicide Bioassays
- Immunoassays

✓ Western Blot



✓ ELISA



✓ Lateral Flow Sticks

High technological Methods

- RNA-based methods
- DNA-based methods

✓ Qualitative PCR



✓ Multiplex PCR

✓ Quantitative PCR: Real-time PCR

✓ Microarrays



Protein-based Methods for Detection of GMOs

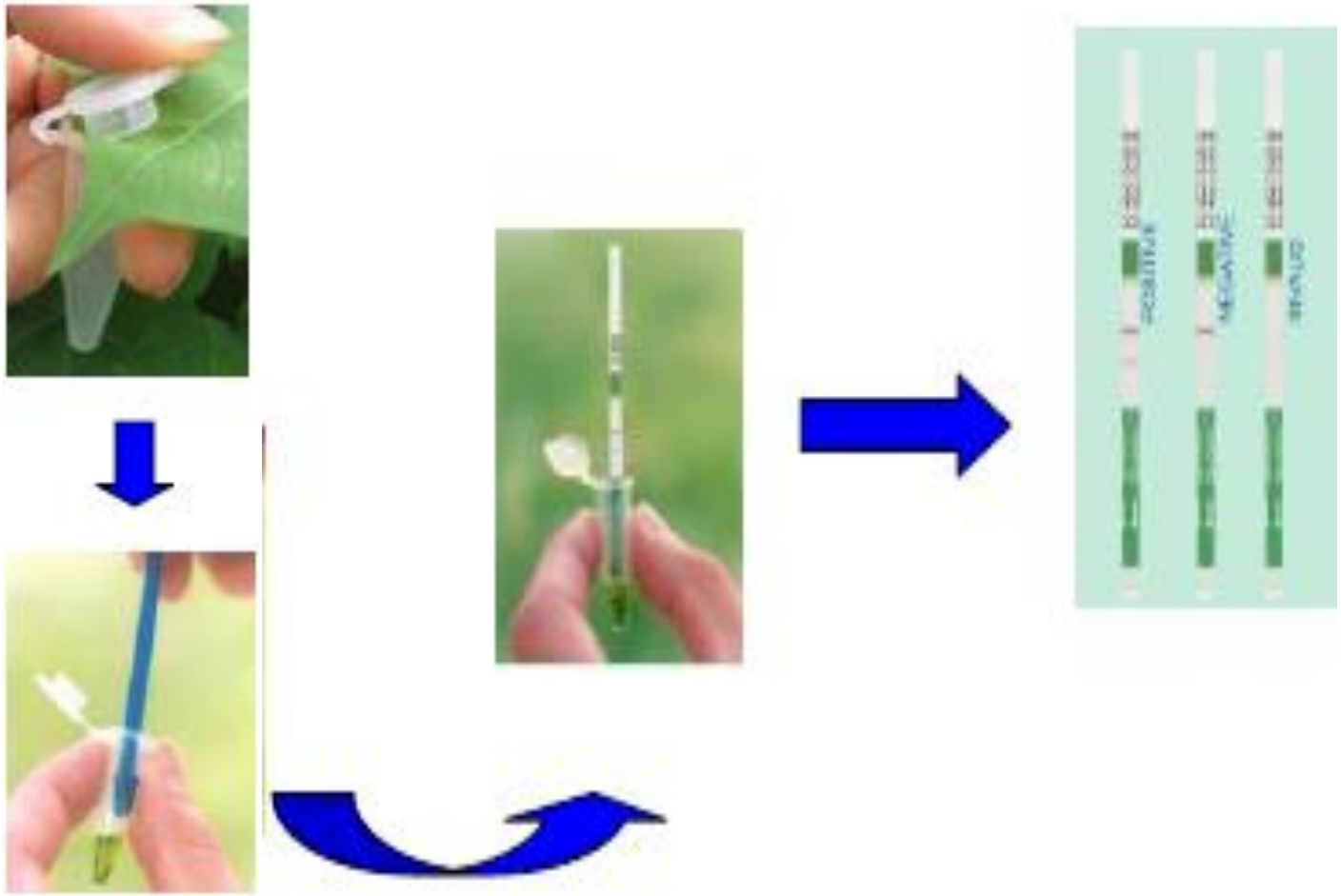
1. Lateral Flow Strip Methods
2. Enzyme Linked Immunosorbent Assay (ELISA)

Protein based methods

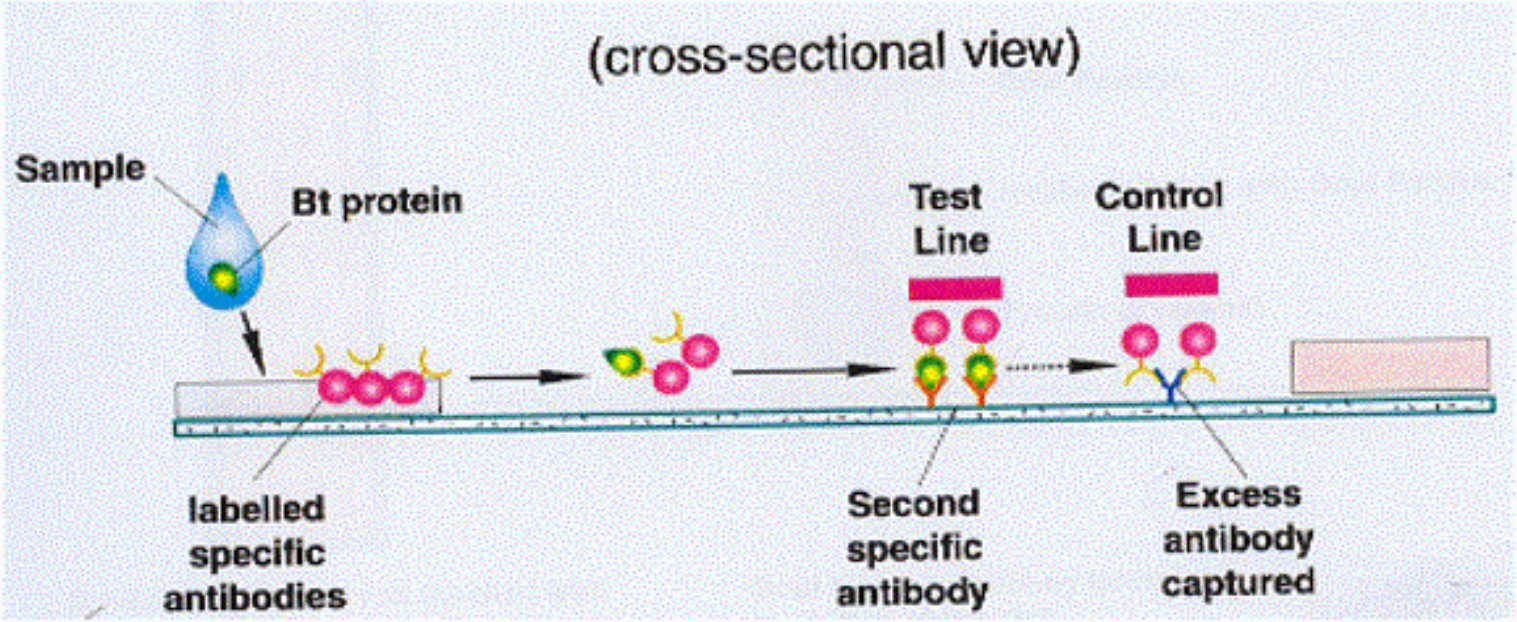


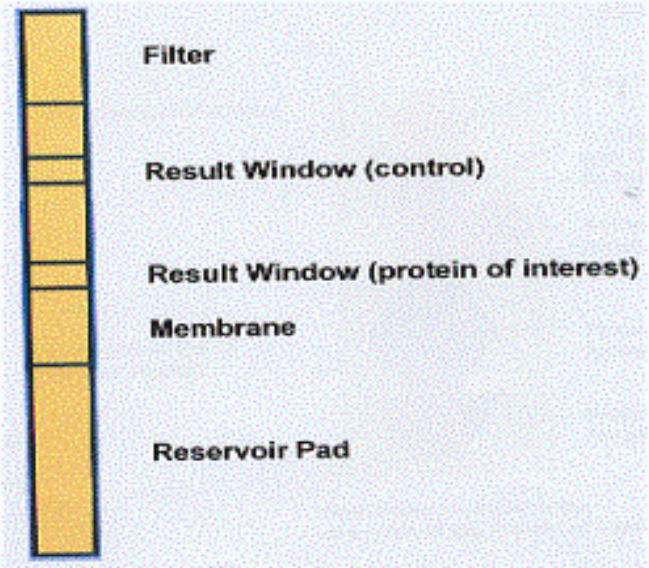
- ✓ Rapid turnaround - 5 to 20 mins for strips; 24 hours for ELISA
- ✓ Relatively cheap to perform
- ✓ Strips do not require trained personnel
- ✓ Limited to one or a small number of varieties per Test
- ✓ Not appropriate for processed products
- ✓ Not appropriate for some GM varieties - in certain crops the GM protein is only produced in the leaves or stems and not in the actual grain. Protein tests on the grain are therefore not informative

Lateral Flow Strip Method



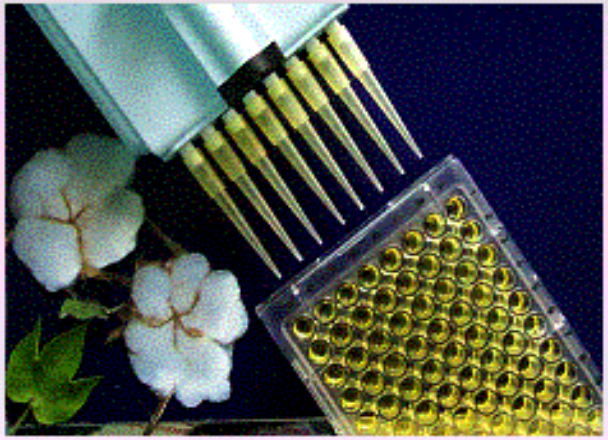
Lateral Flow Strip





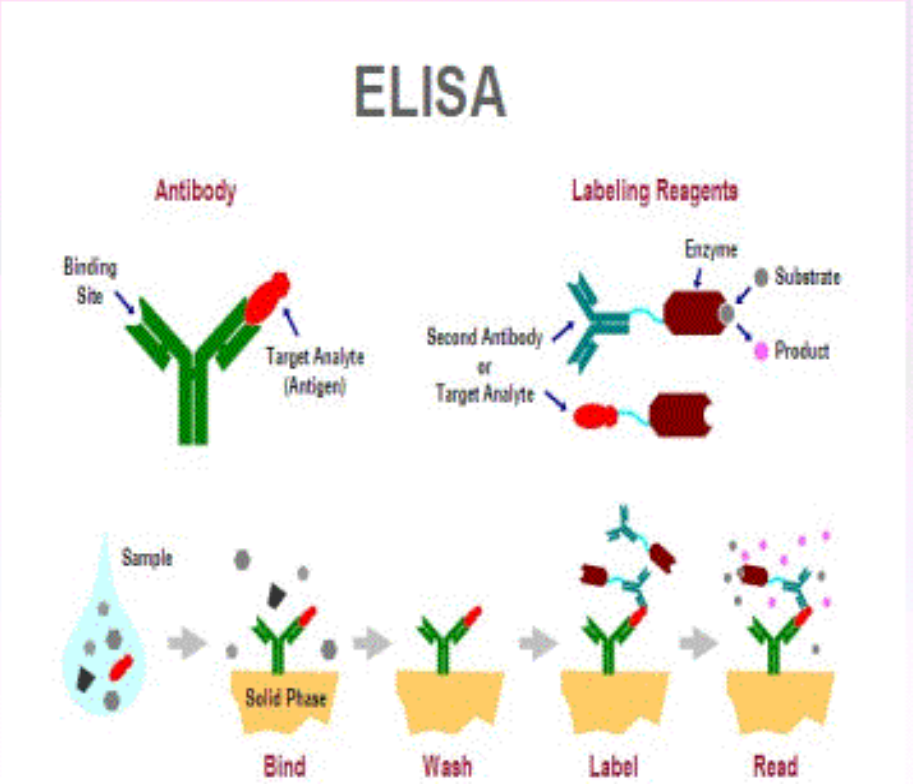
Protein based methods

Enzyme-Linked Immunosorbent Assay (ELISA)



- Specific Antibodies
- Positive control
- Negative control
- Buffer control
- Each sample in duplicate wells

Principle



Advantages of ELISA

Reasonably sensitive

Less susceptible to 'false positives'

Low per sample cost

Handles large number of samples

Can be subjected to automation

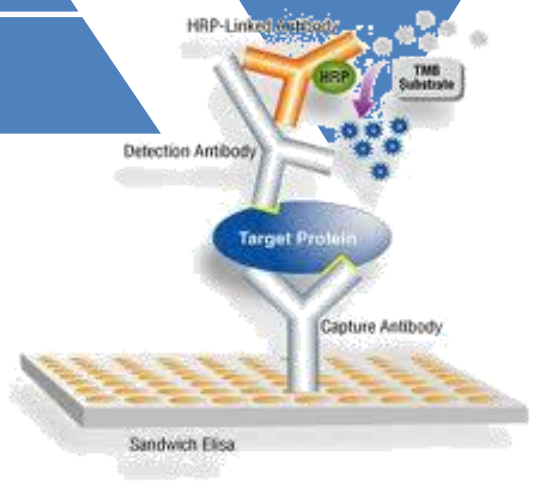
Detection kits available commercially

Limitations of ELISA

GM products might be produced only during certain developmental stages, so, difficult to be detected

In processed foods the proteins denatures easily

High development costs for the assays





DNA based methods

- ✓ Highly sensitive-can detect trace amounts of recombinant DNA
- ✓ Work with most product types:

  both processed and unprocessed products
- ✓ Can test for multiple GM events/varieties simultaneously
- ✓ Takes a little longer to perform the tests
- ✓ Requires highly skilled personnel and laboratory analysis
- ✓ More expensive than Protein Based Methods

Strategies for DNA-based detection

PCR-based

Conventional PCR
Amplification of DNA segment using specific primers

Multiplex PCR
Screening & detection of several target sequence in single reaction

Quantitative Competitive-PCR
Band intensity of target sequence compared with an internal standard

Real-time PCR
Allows monitoring of amplification reaction as it actually occurs.(i.e. in real time)

Isothermal amplification based

- Visual LAMP
- Real-time LAMP

Hybridization-based

- Southern Hybridization
- GMO chip

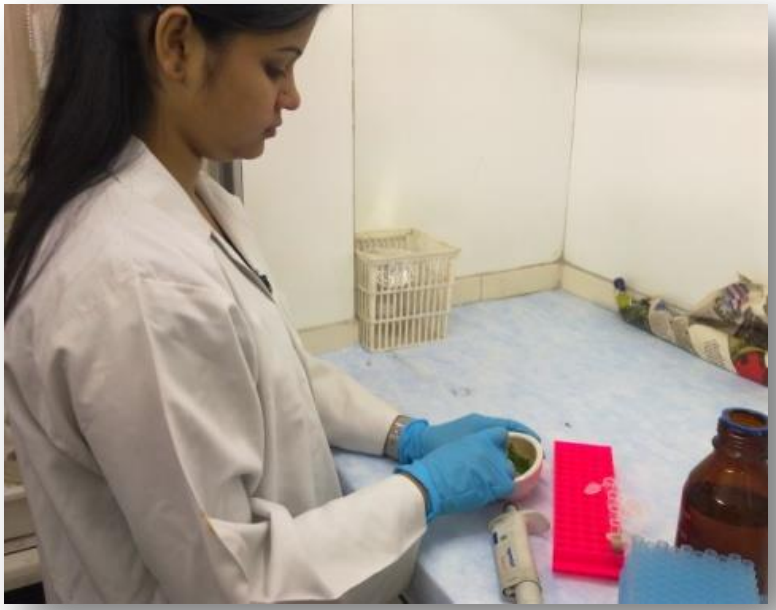
DNA Extraction

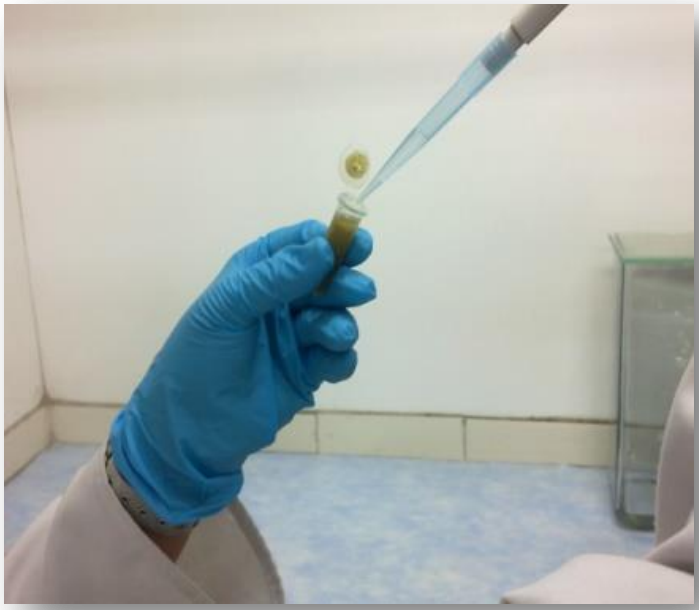
Isolation of Genomic DNA (containing the gene of interest) from the LMO

Using Conventional Methods (CTAB/SDS Extraction Protocol)

OR

Commercialized Kits for DNA Isolation





DNA Quantification

Extracted DNA is quantified using Nano-Drop UV-Spectrophotometer

↓

To check the quantity and quality of DNA

↓

Proceed for Polymerase Chain Reaction

Primer Designing

DNA target sequence

Gggttgttgtgtatgcagtcagaaaagagagacgaatgggtgcttctttgctacgt
ttacattttcatgattgtttcgttaacggttgtgatgcttcgattctccttgaccaaacttc
taccattaatagtgaaaagacttctcg**7th Indo-Global Summit and Expo on Food and Beverages**aacaattctgctagaggatttgaggtgattgataaaattaaatca
gaggttgataaagtttgtggacgtccgggggttgttgtgtatgcagtcagaaaag
agagacgaatgggtgcttctttgctacgtttacattttcatgattgtttcgttaacggtt
gtgatgcttcgattct

Primers

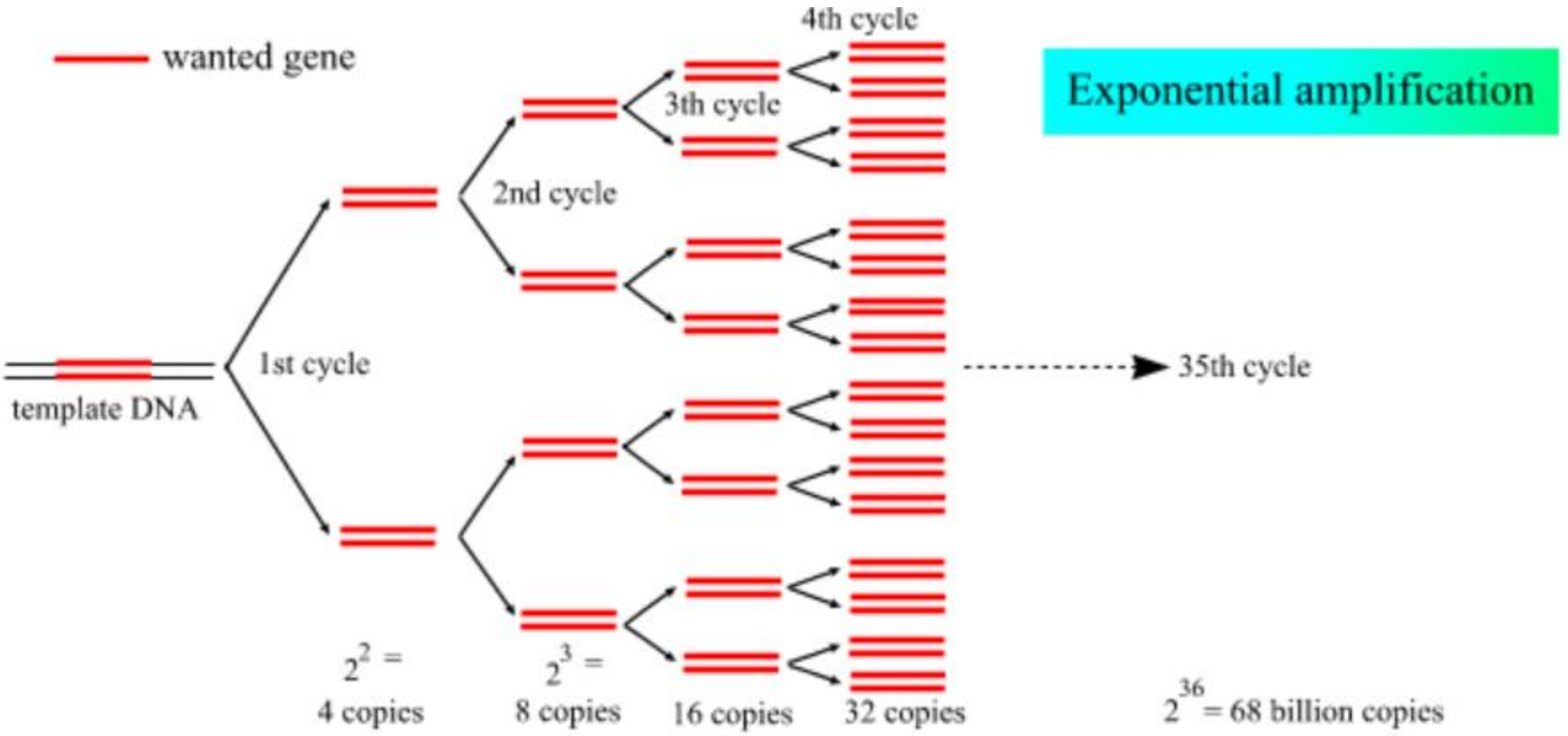
Tgatgcttcgattctccttgaccaaacttctaccattaatagtg

aaaagacttctcgtgctaactgaggcaatagcattgctaactta

acgatt attga

acaattctgctagaggatttgaggtgattgataaaattaaat

Polymerase Chain Reaction



(Andy Vierstraete 1999)

PCR BASED DETECTION

- **Detects a known DNA sequence by amplification**
- **Target sequence is duplicated billions of times**
- **Results obtained can be quantitative or qualitative**
- **Amplification is done in changing temperature cycles**
- **Enzyme :Taq polymerase**
- **Equipment : Thermocycler/PCR machine**







PCR Set up

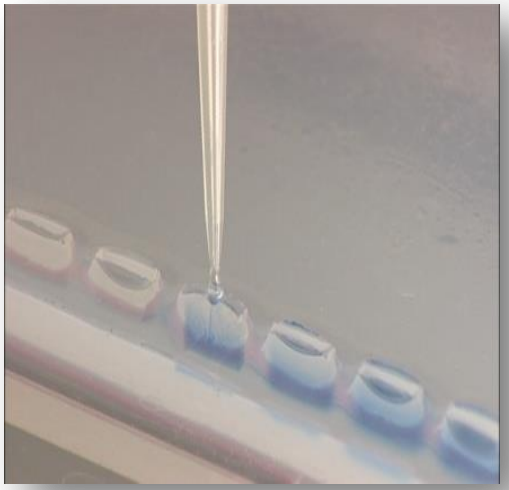




PCR Programming



Analysis of PCR Products



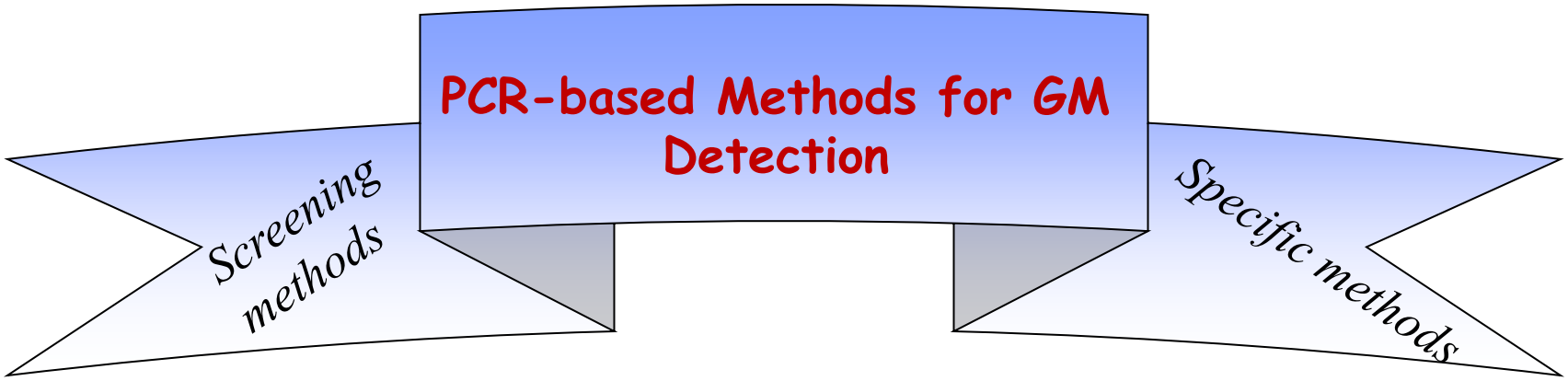
Agarose Gel Electrophoresis



Analysis of PCR amplicons on Gel Documentation System

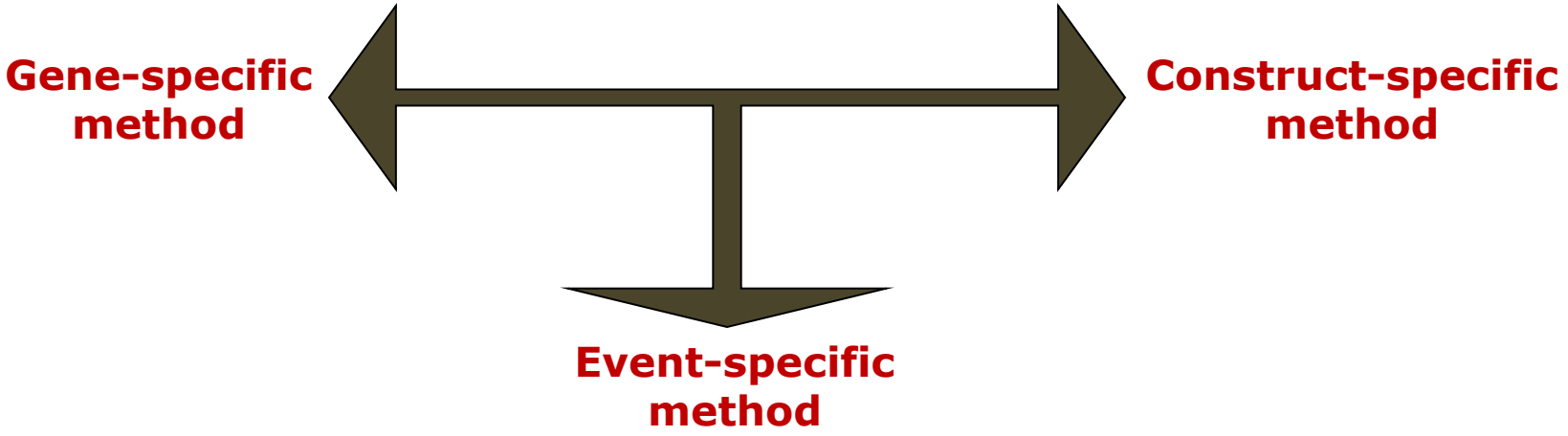


PCR-based Methods for GM Detection



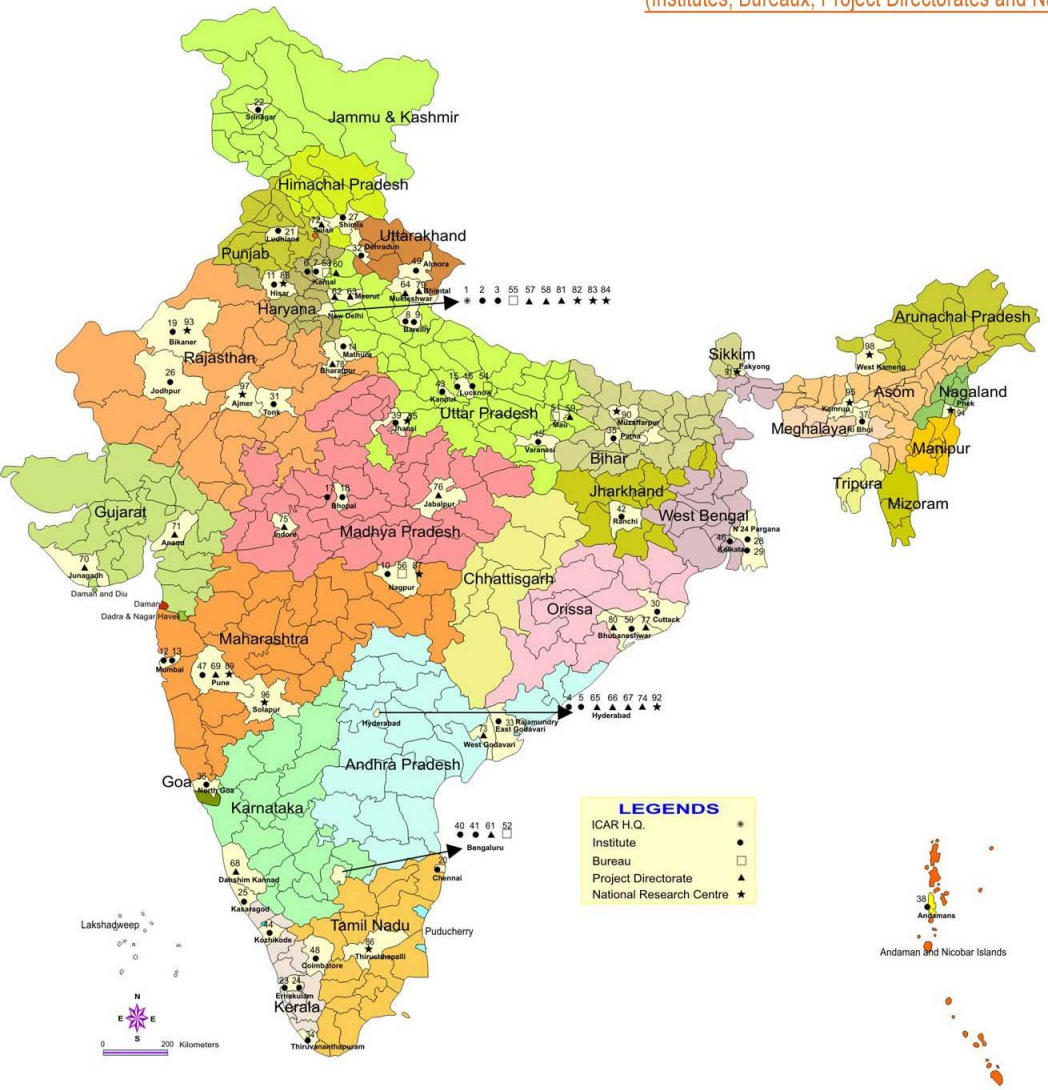
- First tool for the analysis of unknown samples
- Allowing to detect sequences that are commonly found in transgenic events, in particular, some promoters, terminators and selectable marker genes

- Useful for confirming positive results obtained with screening methods
- Allow the tracking of events within the seed company during development of the variety



Indian Council of Agricultural Research

(Institutes, Bureaux, Project Directorates and National Research Centres)



Entity ID	Headquarters
1	Indian Council of Agricultural Research, New Delhi

	ICAR Institutes
2	Indian Agricultural Research Institute, New Delhi
3	Indian Agricultural Statistics Research Institute, New Delhi
4	National Academy of Agricultural Research Management, Hyderabad, Andhra Pradesh
5	Central Research Institute for Dryland Agriculture, Hyderabad, Andhra Pradesh
6	Central Soil Salinity Research Institute, Karnal, Haryana
7	National Dairy Research Institute, Karnal, Haryana
8	Central Avian Research Institute, Bareilly, Uttar Pradesh
9	Indian Veterinary Research Institute, Bareilly, Uttar Pradesh
10	Central Institute for Cotton Research, Nagpur,
11	Central Institute for Research on Buffaloes, Hisar, Haryana
12	Central Institute for Research on Cotton Technology, Mumbai, Maharashtra
13	Central Institute of Fisheries Education, Mumbai
14	Central Institute for Research on Goats, Mathura, U.P.
15	Indian Institute of Sugarcane Research, Lucknow, U.P.
16	Central Institute for Subtropical Horticulture, Lucknow, U.P.
17	Central Institute of Agricultural Engineering, Bhopal, M.P.
18	Indian Institute of Soil Science, Bhopal, Madhaya Pradesh
19	Central Institute for Arid Horticulture, Bikaner, Rajasthan
20	Central Institute of Brackishwater Aquaculture, Chennai
21	Central Institute of Post Harvest Engineering & Technology, Ludhiana, Punjab
22	Central Institute of Temperate Horticulture, Srinagar, J&K
23	Central Marine Fisheries Research Institute, Cochin
24	Central Institute of Fisheries Technology, Cochin
25	Central Plantation Crops Research Institute, Kasaragod
26	Central Arid Zone Research Institute, Jodhpur, Rajasthan
27	Central Potato Research Institute, Shimla, H.P.
28	Central Research Institute for Jute and Allied Fibres, Barrackpore, West Bengal
29	Central Inland Fisheries Research Institute, Barrackpore
30	Central Rice Research Institute, Cuttack, Orissa
31	Central Sheep and Wool Research Institute, Avikanagar
32	Central Soil and Water Conservation Research and Training Institute, Dehradun, Uttarakhand
33	Central Tobacco Research Institute, Rajamundry, A.P.
34	Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala
35	ICAR Research Complex for Eastern Region, Patna, Bihar
36	ICAR Research Complex for Goa, Goa
37	ICAR Research Complex for NEH Region, Ri Bhoi,
38	Central Agricultural Research Institute, Port Blair, A&N
39	Indian Grassland & Fodder Research Institute, Jhansi, U.P.
40	Indian Institute of Horticultural Research, Bengaluru
41	National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka
42	Indian Institute of Natural Resins and Gums, Ranchi
43	Indian Institute of Pulses Research, Kanpur, U.P.
44	Indian Institute of Spices Research, Marikunnu, Kerala
45	Indian Institute of Vegetable Research, Varanasi, U.P.
46	National Institute of Research on Jute and Allied Fibre Technology, Kolkata, West Bengal
47	National Institute of Abiotic Stress Management, Malegaon, Pune, Maharashtra

Entity ID	ICAR Institutes
48	Sugarcane Breeding Institute, Coimbatore, Tamil Nadu
49	Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand
50	Central Institute of Freshwater Aquaculture, Bhubaneshwar, Orissa

	Bureaux
51	Agriculturally Important Micro Organisms, Mau Nath Bhanjan, Uttar Pradesh
52	Agriculturally Important Insects, Bengaluru, Karnataka
53	Animal Genetic Resources, Karnal, Haryana
54	Fish Genetic Resources, Lucknow, Uttar Pradesh
55	Plant Genetic Resources, New Delhi
56	Soil Survey & Land Use Planning, Nagpur, Maharashtra

	Project Directorates
57	Maize Research, New Delhi
58	Floricultural Research, New Delhi
59	Seed Research, Mau Nath Bhanjan, Uttar Pradesh
60	Wheat, Karnal, Haryana
61	Animal Disease Monitoring and Surveillance, Bengaluru
62	Farming Systems, Meerut, Uttar Pradesh
63	Cattle, Meerut, Uttar Pradesh
64	Foot and Mouth Disease, Mukteshwar, Uttarakhand
65	Poultry, Hyderabad, Andhra Pradesh
66	Oilseeds Research, Hyderabad, Andhra Pradesh
67	Rice Research, Hyderabad, Andhra Pradesh
68	Cashew Research, Dakshina Kannada, Karnataka
69	Onion and Garlic Research, Pune, Maharashtra
70	Groundnut Research, Junagarh, Gujarat
71	Medicinal and Aromatic Plants Research, Anand, Gujarat
72	Mushroom Research, Solan, Himachal Pradesh
73	Oilpalm Research, West Godavari, Andhra Pradesh
74	Sorghum Research, Hyderabad, Andhra Pradesh
75	Soybean Research, Indore, Madhya Pradesh
76	Weed Science Research, Jabalpur, Madhya Pradesh
77	Women in Agriculture, Bhubaneshwar, Orissa
78	Rapeseed-Mustard Research, Bharatpur, Rajasthan
79	Cold Water Fisheries, Bhimtal, Uttarakhand
80	Water Management, Bhubaneshwar, Orissa
81	Information and Publications of Agri., Pusa, New Delhi

	National Research Centre
82	Agricultural Economics and Policy, New Delhi
83	Integrated Pest Management, New Delhi
84	Plant Biotechnology, New Delhi
85	Agroforestry, Jhansi, Uttar Pradesh
86	Banana, Thiruchirapalli, Tamil Nadu
87	Citrus, Nagpur, Maharashtra
88	Equines, Hisar, Haryana
89	Grapes, Pune, Maharashtra
90	Litchi, Muzaffarpur, Bihar
91	Orchids, Gangtok, Sikkim
92	Meat, Hyderabad, Andhra Pradesh
93	Camel, Bikaner, Rajasthan
94	Mithun, Dimapur, Nagaland
95	Pig, Guwahati, Asom
96	Pomegranate, Solapur, Maharashtra
97	Seed Spices, Ajmer, Rajasthan
98	Yak, West Kameng, Arunachal Pradesh

- 49 ICAR Institutes • 6 Bureaux • 25 Project Directorates • 17 National Research Centres
- 78 AICRPs/AINPs • 589 Krishi Vigyan Kendras (KVK) • 46 State Agricultural Universities (SAUs)
- One Central Agricultural University and 4 Central Universities having faculty of agriculture

ICAR-National Bureau of Plant Genetic Resource New Delhi

Nodal institute for import permit, quarantine processing and issue of phytosanitary certificate for germplasm/transgenic planting material



Facilities for GM detection





CL-4 Containment Facility

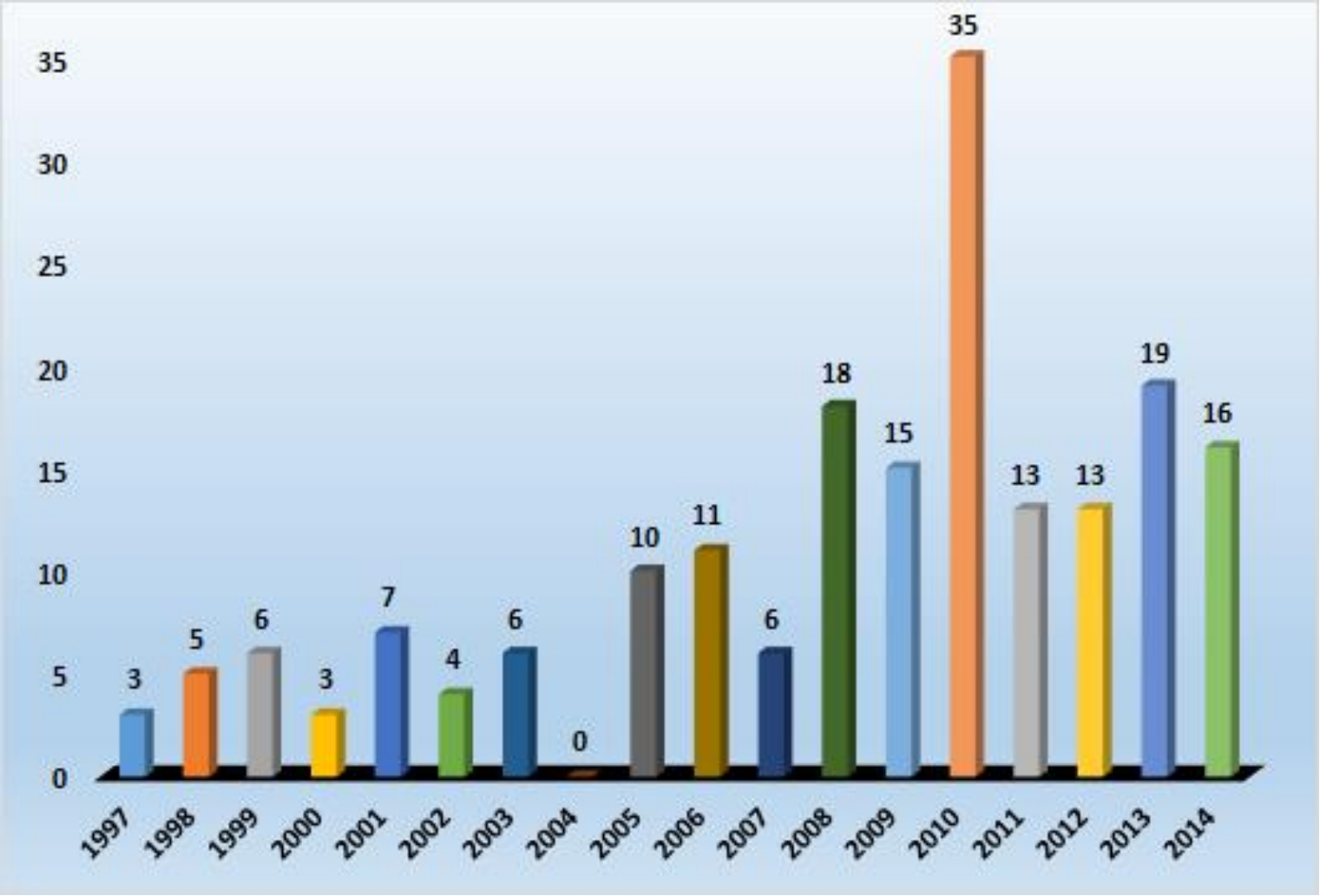




GM Detection Lab

Import of Transgenic Planting Material for Research Purposes through ICAR-NBPGR

So far, 190 imported consignments of 12 crops comprising more than 11, 000 accessions have been introduced.



Containment Facility for Evaluation of Transgenics



Containment Facility



Bollgard III Cotton
(MON15985 x COT102
Importer: Mahyco, New Delhi

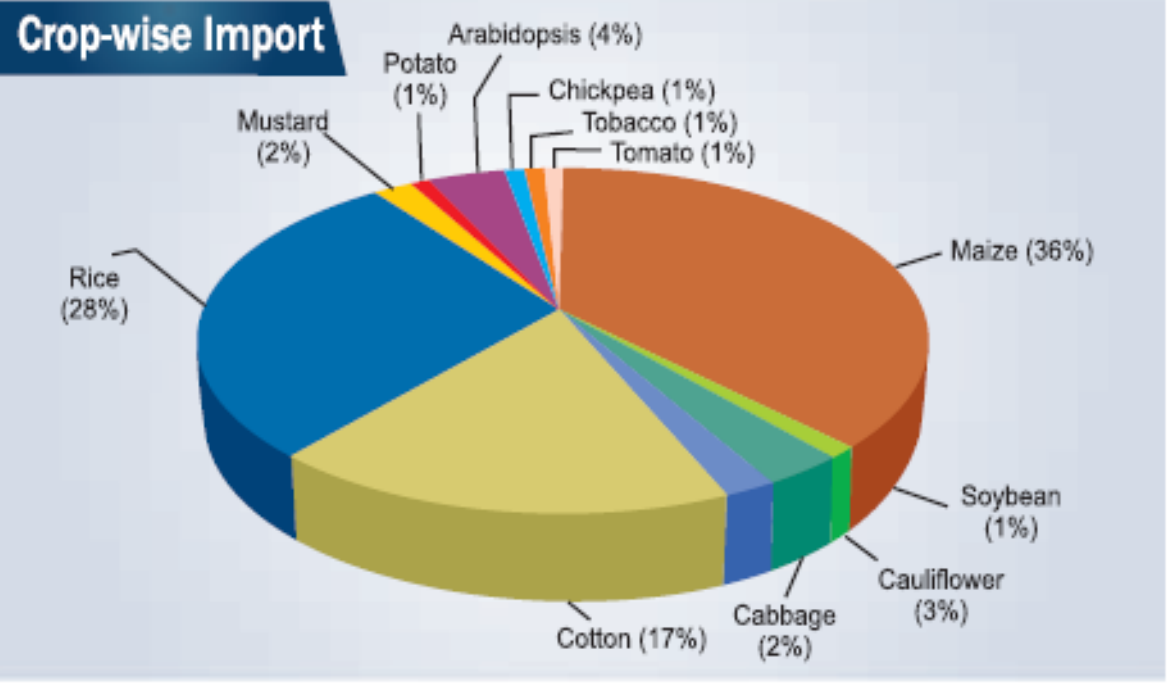


Maize
TLT0766_Bt11 x GA21
Importer: Syngenta BioSciences Pvt. Ltd., Pune



Rice
RPD37-34 events, RPD 38-32 events
Importer: BASF India Limited, Mumbai

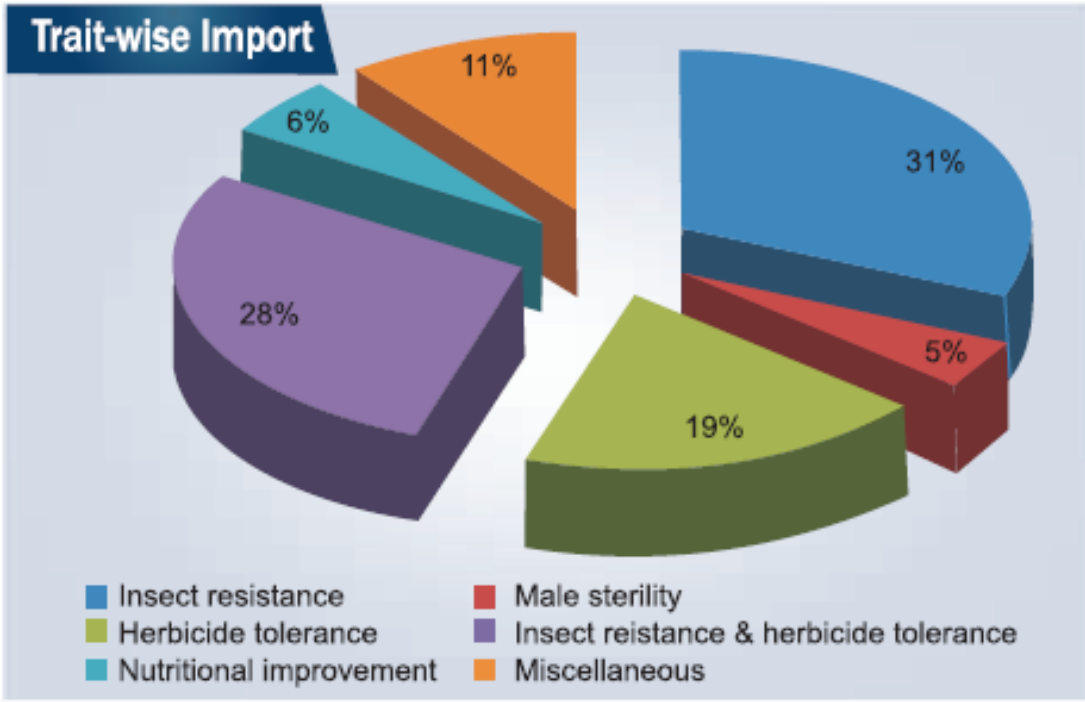
Crop-wise Import







Trait-wise Import

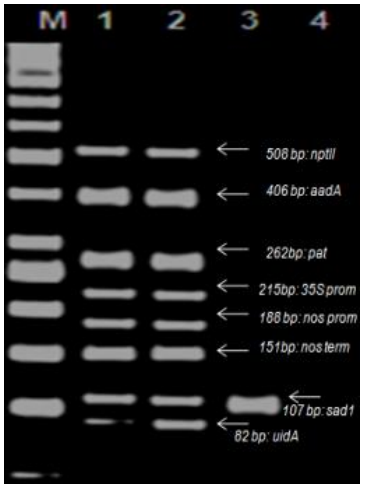


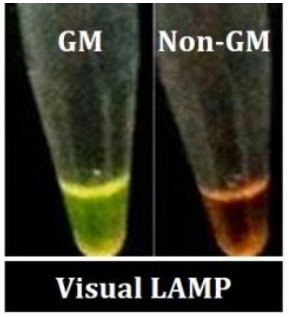
Expertise for Cost/Time-Efficient GMO Screening

- **Singleplex and Multiplex PCR-based screening method:** targeting commonly employed promoters, terminators, marker genes;

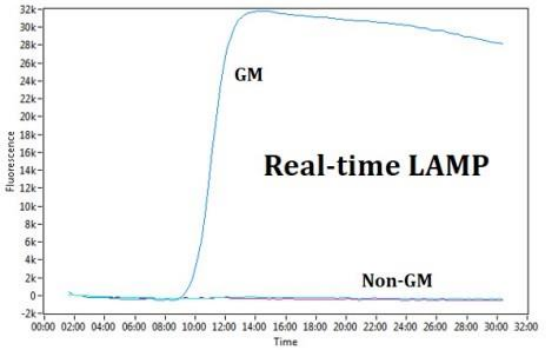
- **GMO screening matrix developed to check authorized and unauthorized GM events in India:** The information on 106 transgenic elements present in 141 events of 21 GM crops (commercialized or imported for research purposes or under field trials in India) has been complied for the GMO matrix. Ten most frequently present targets, have been identified to screen these events;

- **Rapid, cost-effective and reliable GMO screening assays based on visual and real-time LAMP** targeting commonly employed promoters (*P-35S*, *P-FMV*), marker genes (*aadA, nptII*, *uidA*) and transgenes (*cry1Ac, cry2Ab2, cp4-epsps*), **to facilitate on-site GMO screening**, if combined with a fast DNA extraction method.



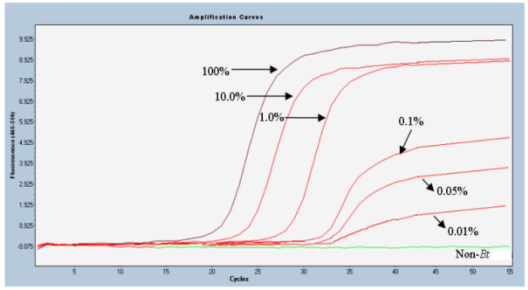


LAMP assays for LMO detection



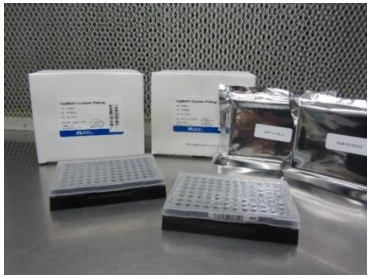
Expertise for GMO Detection and Quantification

- **PCR/Real-time-based GM diagnostics assays** (Transgene/ construct/ event-specific) for more than **40 GM events of 12 GM crops;**
- PCR and LAMP assays for detection and quantification of **commercially approved *Bt* cotton events in India;**
- LAMP assays for **six imported GM maize events;**
- **User-friendly multi-target TaqMan® real-time PCR-based screening system**, simultaneously targeting 47 targets, including 21 GM events, 5 constructs, 8 transgenes, 3 marker genes, 4 control elements and 6 endogenous genes to test an unknown sample for these targets in duplicate;
- **Molecular testing of imported GM planting material:** All **190 consignments of 12 GM crops imported** for research purposes through NBPGR were tested;
- **Monitoring adventitious presence of transgenes** in *ex-situ/in-situ* collections of brinjal, cotton, and okra.



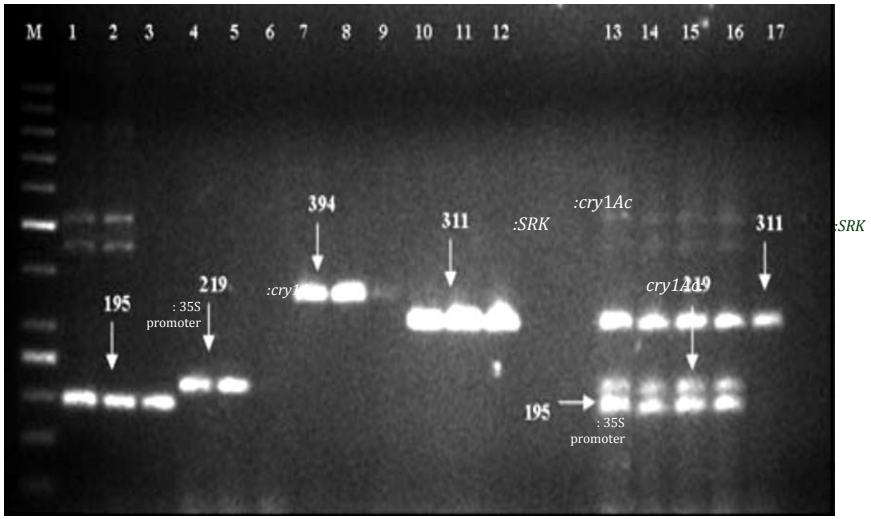






Standardization and validation of endogenous genes in GM crops

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*
Wheat	*β-puroindoline*



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

Initial screening through simplex/multiplex PCR targeting marker and reporter genes: To test the GM status of a sample irrespective of crop & GM trait

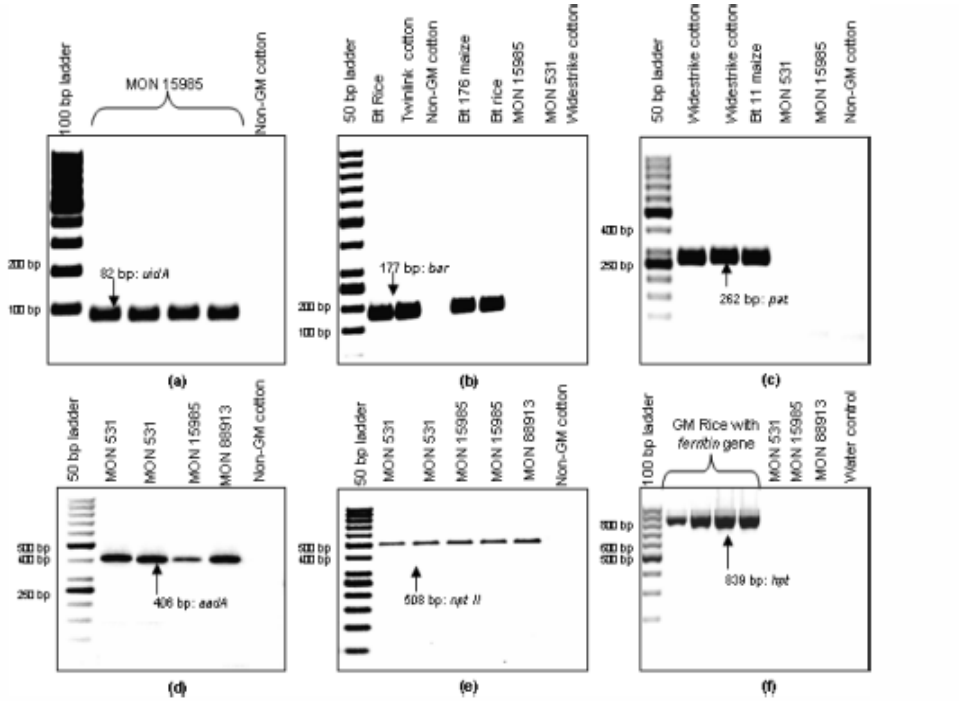


Figure 1. Simplex PCR amplicons in different transgenic seed materials for their respective selectable marker genes using the specific primer pairs for the detection of (a) *uidA*; (b) *bar*; (c) *pat*; (d) *aadA*; (e) *npt II*; and (f) *hpt* genes.

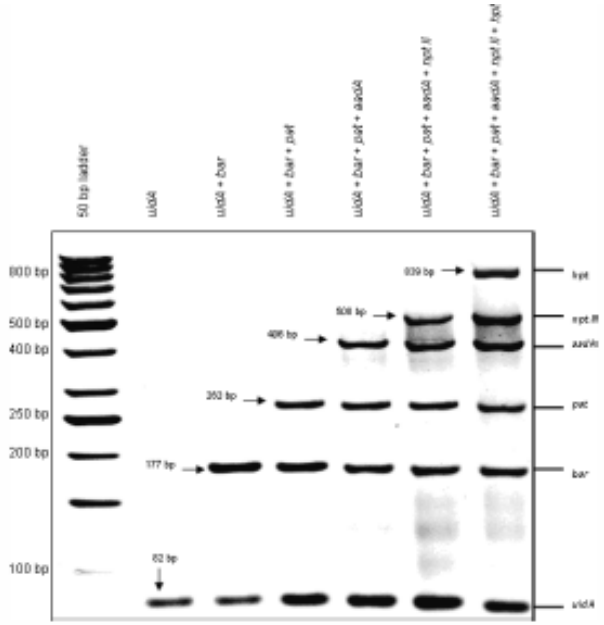
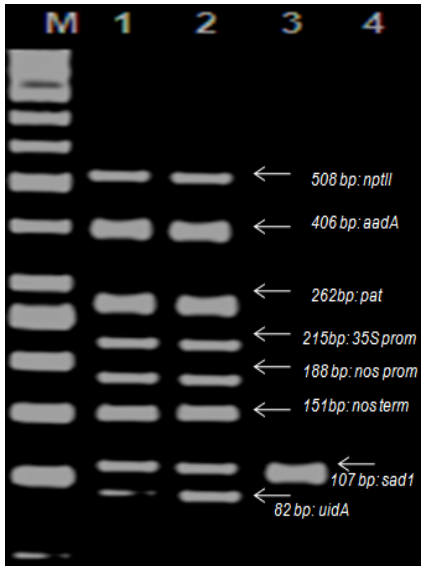


Figure 2. Multiplex PCR assay for testing of primer interference using equivalent DNA mix of six different GM events, i.e., MON 531 of cotton, MON 15985 of cotton, Widestrike cotton, Bt rice, GM rice with the fermitin gene and Bt176 of maize.

Simultaneous amplification of six commonly used marker genes
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 cotton



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), Lane 3: Non-GM cotton, Lane 4: NTC

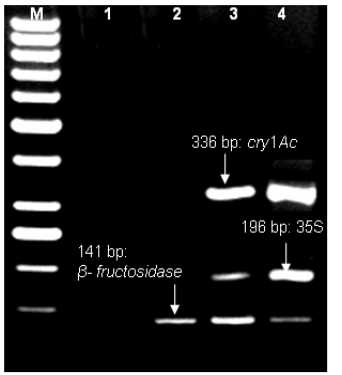
Simultaneous amplification of seven targets

Control elements: *P-35S*, T-*nos*, P-*nos*

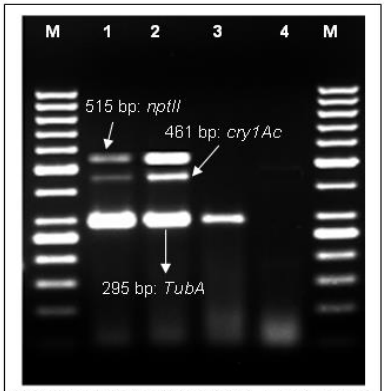
Marker genes: *nptII*, *aadA*, *pat* and *uidA*

Endogenous gene: *Sad1*

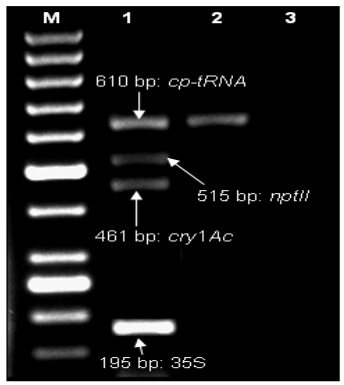
Gene-specific PCR



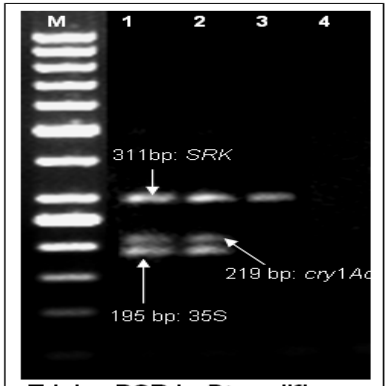
Triplex PCR in *Bt* brinjal with *cry1Ac* gene
1: Water control
2: Non-*Bt* brinjal
3-4: *Bt* brinjal
M: 50bp DNA ladder



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

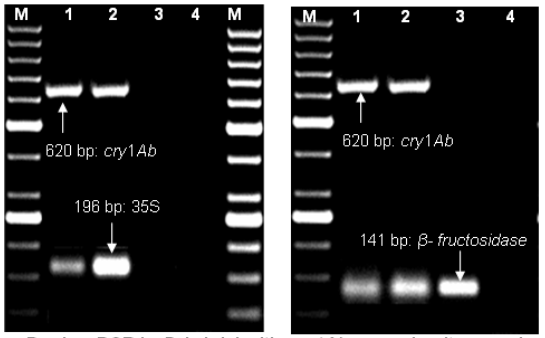


Tetraplex PCR in *Bt* okra with *cry1Ac* gene
1: *Bt* okra
2: Non-*Bt* okra
3: Water control
M: 50bp DNA ladder

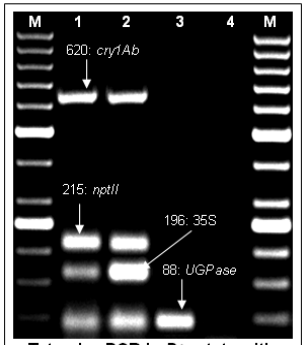


Triplex PCR in *Bt* cauliflower with *cry1Ac* gene
1-2: *Bt* cauliflower
3: Non-*Bt* cauliflower
4: Water control
M: 50bp DNA ladder

Targeting the specific genes (*cry1Ac, cry1Ab*) in the construct



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

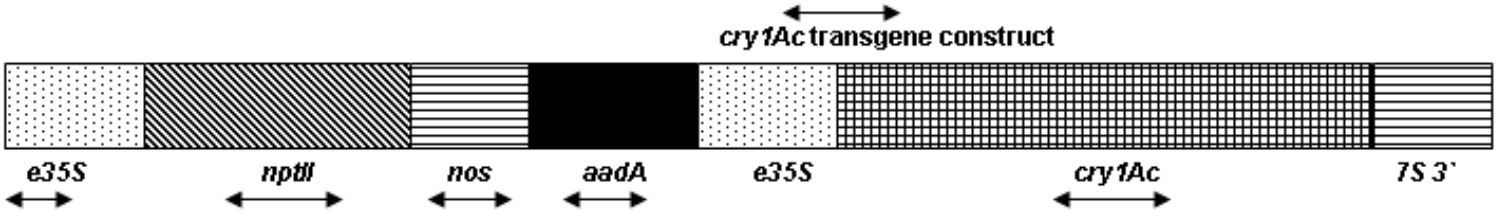


Tetraplex PCR in *Bt* potato with *cry1Ab* gene
1-2: *Bt* potato
3: Non-*Bt* potato
4: Water control
M: 50bp DNA ladder

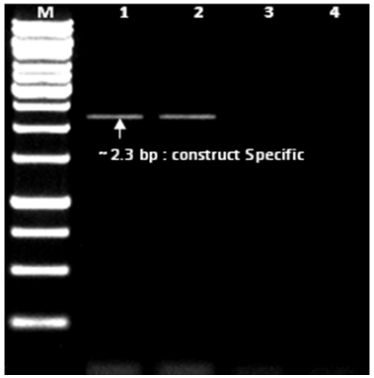
GJ Randhawa, M Singh, R Chhabra and R Sharma (2010) Qualitative and quantitative molecular testing methodologies and traceability systems for *Bt* crops commercialised or under field trials in India. *Food Analytical Methods* 3 (4), 295-303

Construct-specific PCR

Targeting the junction region of two elements of construct

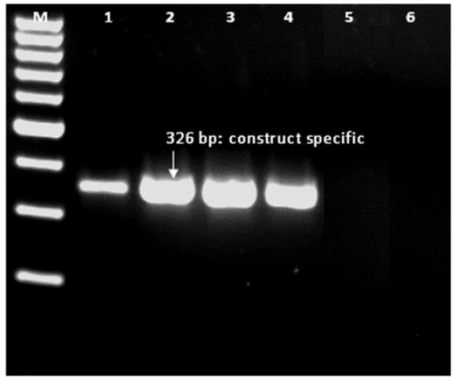


Linear Transgene Construct of MON 531



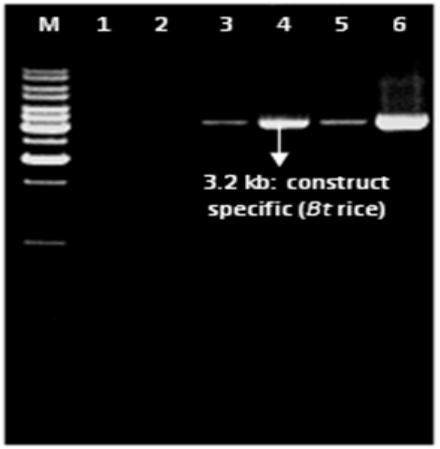
Construct-specific PCR for detection of a part of inserted gene construct in GM Tomato (*avp1* gene)

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

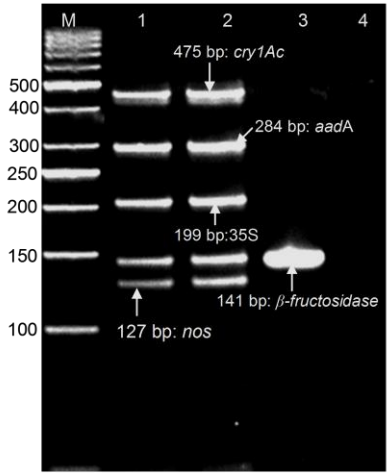
Lane 1: BGI, Lane 2: BGII, Lane 3: Event1, Lane 4: GFM-cry1A
Lane 5: Non-GM cotton, Lane 6: Water control, M: 100 bp ladder



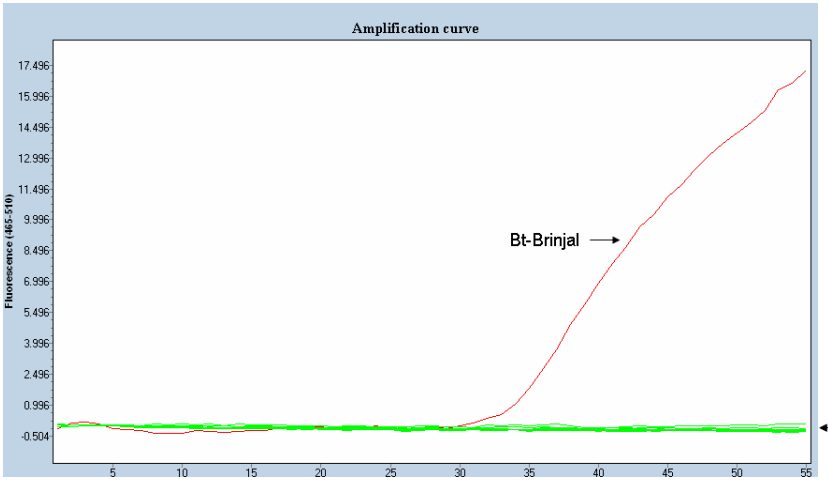
Construct-specific PCR for detection of a part of inserted gene construct in GM rice (*cry1Ac* gene)

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

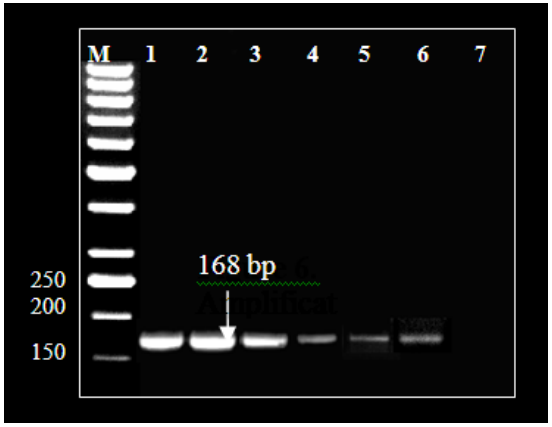
Event-specific detection of *Bt* brinjal event EE-1



Pentaplex PCR detection of *Bt* brinjal event EE-1, M: 50 bp ladder; Lanes 1, 2: Samples of *Bt* brinjal; Lane 3: Sample of non-*Bt* brinjal; Lane 4: Water control



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

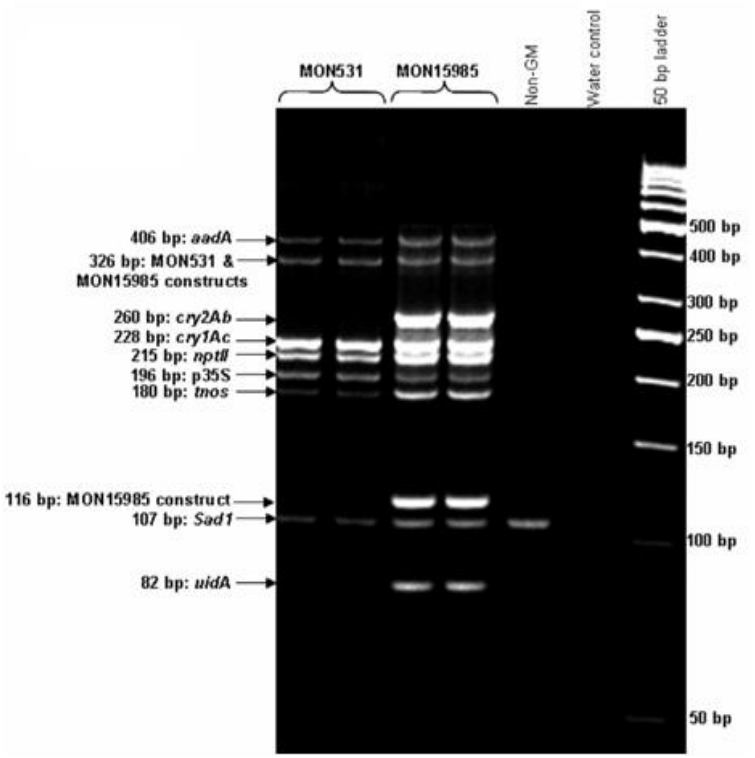


Lane M: 50bp ladder; Lane M: 1kb ladder, Lanes 1-6: Serial dilutions of *Bt* brinjal with 100, 10, 1.0, 0.1, 0.05, 0.01% of GM content, Lane 7: Non-*Bt* brinjal

LOD is upto 0.01%

GJ Randhawa, R Sharma & M Singh (2012) Qualitative and event-specific real-time PCR detection methods for *Bt* brinjal event EE-1. *J. AOAC Int.* 95 (6): 1733-1739

Molecular Diagnostics for Commercialized *Bt* Cotton Events



Transgene- and construct-specific multiplex PCR for discrimination of two *Bt* cotton events, 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



insect infestation on Bt (right) and non-Bt (left) cotton bolls

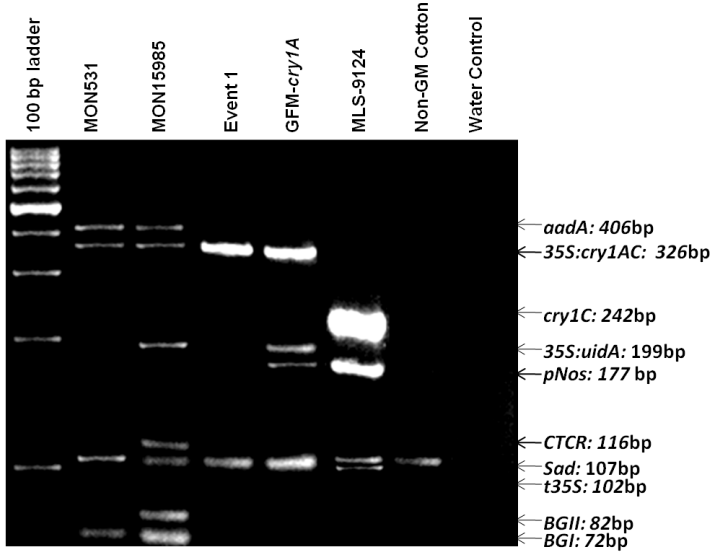
Randhawa G.J., R Chhabra and M Singh (2010) Decaplex and Real-Time PCR Based Detection of MON531 and MON15985 Bt cotton events. J. Agri. Food Chem. (2010) 58 (18), 9875–9881

Molecular Diagnostics for Commercialized *Bt* Cotton Events

Area under commercial cultivation of GM crops: 11.6 mha

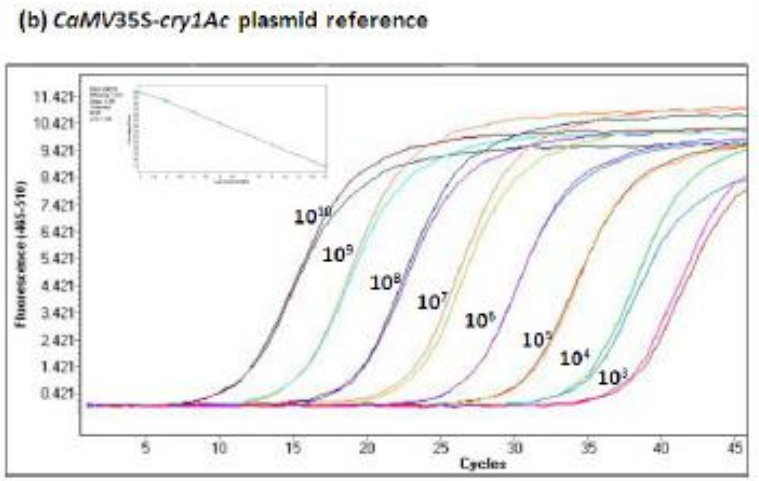
Transgenic elements of five commercialized GM cotton events employed in decaplex PCR to detect these events

GM cotton Event	*T35S*	*Pnos*	*aadA*	*P35S-uidA*	*P35S-cry1Ac*	*CTP-cry2Ab*	*cry1C*	MON531 event	MON 15985 event
MON531			✓		✓			✓	
MON15985			✓	✓	✓	✓		✓	✓
Event 1					✓				
GFM-cry1A		✓		✓	✓				
MLS-9124	✓	✓					✓		



Limit of Detection (LOD): Upto 0.01%

***P35S- cry1Ac* Construct-specific Real-time PCR for quantification of four major GM events**



Source: R Chhabra, GJ Randhawa GJ, RK Bhoge & M Singh (2014) Qualitative and quantitative PCR-based detection methods for authorized genetically modified cotton events in India. J. AOAC Int. 97 (5): 1299-1309

Simplex/Multiplex PCR assays developed/validated

GM Crop/Event	Trait	Transgenic elements	Species/taxon-specific endogenous gene
Cotton			
MON531	IR	*cry1Ac, nptII, aadA, P-35S, T-nos*	*Sad1/fs-ACP*
MON15985	IR	*cry1Ac, cry2Ab2, nptII, aadA, uidA, P-35S, T-nos*	
Event1	IR	*cry1Ac, P-35S, T-nos*	
GFM-cry1A	IR	*cry1Ab-Ac, nptII, P-35S, T-nos*	
MLS-9124	IR	*cry1C, P-nos, T-35Ss*	
Widestrike™	IR	*cry1F, cry1Ac, P-ubiq, T-nos*	
MON1445 (Roundup Ready)	HT	*cp4-epsps, P-35S, T-nos*	
MON15985 x MON88913 (*Bt* Roundup Ready Flex)	IR, HT	*cry1Ac, cry2Ab2, cp4-epsps, P-35S, T-nos*	
MON88913 (Roundup Ready Flex)	HT	*cp4-epsps, P-35S, T-nos*	
Brinjal			
EE1	IR	*cry1Ac, nptII, aadA, P-35S, T-nos*	*β-fructosidase*
Bt* brinjal with *cry1Ab	IR	*cry1Ab, P-35S, T-nos*	
Event142	IR	*P35S-cry1Ab construct-specific* *Cry1Fa1, P-35S* *P35S-cry1Fa1 construct-specific*	

Simplex/Multiplex PCR assays developed/validated

GM Crop/Event	Trait	Transgenic elements	Species/taxon-specific endogenous gene
Maize			
MON810	IR	*cry1Ab, hsp70, P-35S, T-nos*	
Bt11	IR	*cry1Ab, bar, P-35S, T-nos*	
Bt176	IR	*cry1Ab, pat, P-35S, T-nos*	
TC1507	IR	*cry1F, pat, P-35S, P-ubiq, T-nos*	
GA21	HT	*m-epsps, P-35S, P-ract, T-nos*	
NK603	HT	*cp4-epsps,hsp70, nptII, P-35S, P-ract, T-nos*	
MON89034	IR	*Cry1A.105, cry2Ab2, nptII, P-FMV, T-nos*	
Bt11 x GA21	IR, HT	*P-35S, P-ract, pat, T-nos*	
MON89034 x NK603	IR, HT	*cry1A.105, cry2Ab2, cp4-epsps, hsp70, nptII, P-35S, P-FMV, T-nos*	*Adh1/hmg*
TC1507 x NK603	IR, HT	*cry1F, cp4-epsps, hsp70, nptII, P-35S, P-ract, T-nos*	
TC1507 x MON810	IR	*Cry1F, hsp70, pat, nptII, P-35S, T-nos* *Bt11, MON810, NK603, GA21, MON89034, TC1507 : Event-specific hexaplex PCR*	
Tomato			
GM tomato with *osmotin* gene	DST	*osmotin, P-35S*	*LAT52*
GM tomato with *AVP1D* gene	DST	*AVP1D, nptII, P-35S, T-nos*	*β-fructosidase*
Rice			
MRP501*Bt* hybrid	IR	*cry1Ac, nptII, P-35S, T-nos* *P35S-cry1Ac construct-specific*	*α-tubulin*

Simplex/Multiplex PCR assays developed/validated

GM Crop/Event	Trait	Transgenic elements	Species/taxon-specific endogenous gene
Okra			
***Bt* okra**	IR	*cry1Ac, nptII, P-35S, T-nos*	*Chloroplast-tRNA*
Potato			
Bt* potato with *cry1Ab	IR	*Cry1Ab, nptII, P-35S, T-nos*	*β-fructosidase/ UGPase*
GM potato with *AmA1*	NE	*AmA1, nptII, P-35S, T-nos*	
Late blight resistant GM potato	DR	*RB, nptII, P-35S, T-nos*	
Cauliflower			
CFE1	IR	*cry1Ac, P35S*	*SRK*
Cabbage			
***Bt* cabbage**	IR	*P35S-cry1Ac construct-specific*	*SRK*
Mustard			
GM mustard	MS	*barnase, barstar, P-35*	*hmg1/γ*
Soybean			
GTS40-3-2	HT	*cp4-epsps, P-35S, T-nos*	*lectin*

IR: Insect resistance, HT: herbicide tolerance, DR : Disease resistance, DST: drought and salinity tolerance, MS : Male sterility, NE: Nutritional enhancement

Development of GMO Matrix (Decision Support System) for Cost-effective Screening of Authorized and Unauthorized GM Events

*Studies for development of **novel cost-effective GM diagnostics** were designed and conducted in collaboration with Slovenian partners*

- GMO screening matrix, **for 141 GM events of 21 crops** with the information on **106 genetic element targets for detection**, was developed;
- The matrix includes commercially cultivated *Bt* cotton events and other GM events, under field trials during the past six years (imported/indigenously developed);
- Ten most frequently present targets, *viz.*, ***P-35S, T-nos, Os-Msca1, cry1Ab, cry1Ac, cry1C, cry2Ab, GA20 oxidase1, nptII, bar*** were identified to screen these events using the **GMOseek algorithm**.

Genetic elements frequently appearing in combinations resulting from the GMO-seek simulations for all plants and the main crop species.

Crops	Status	Genetic elements
All plants	All status	[***P-35S***] [***T-nos***] [***Os-Msca1***] [***cry1Ab***] [***cry1Ac***] [***Cry1C***] [***cry2Ab***] [***GA20 oxidase1***]
	Commercialized	[***P-35S***]
	Field trials	[***P-35S***] [***T-nos***] [***Os-Msca1***] [***cry1Ad***] [***GA20 oxidase1***] [***nptII***]
	Imported	[***P-35S***] [***T-nos***] [*m-epsps*] [***cry1Ab***] [***cry1Ac***] [***cry1C***] [***cry2Ab***] [***bar***] [*gat*]
	Indigenously developed	[***T-nos***] [***Os-Msca1***] [***cry1Ac***] [***cry2Ab***] [*mtID*] [***GA20 oxidase1***] [***nptII***] [***bar***]
Cotton	All status	[***P-35S***] [*m-EPSPS*] [***cry1Ac***] [*cry1F*]
	Commercialized	[***P-35S***]
	Field trials	[***P-35S***] [*m-EPSPS*] [***cry1Ac***] [*cry1F*]
	Imported	[***P-35S***] [*m-EPSPS*] [***cry1Ac***]
	Indigenously developed	[***T-nos***] [***cry1Ac***]
Rice	All status	[***P-35S***] [***T-nos***] [***cry1Ab***] [***cry1Ac***] [***cry1C***] [***cry2Ab***] [*gat*]
	Commercialized	/
	Field trials	[***T-nos***] [***cry1Ab***] [***cry2Ab***] [cry2Ad]
	Imported	[***P-35S***] [***cry1Ab***] [***cry1Ac***] [***cry1C***] [***cry2Ab***] [*gat*]
	Indigenously developed	[***cry1Ac***] [***cry2Ab***]
Corn	All status	[***P-35S***]
	Commercialized	/
	Field trials	[***P-35S***]
	Imported	[***P-35S***]
	Indigenously developed	/

Source: Randhawa GJ, Morisset D, Singh M & Žel J (2014) GMO matrix: A cost-effective approach for screening unauthorized GM events in India. *Food Control*, 38: 124-129

Practical Utility of GMO Matrix

- Most efficient and cost-effective strategy to screen authorized GM events or adventitious presence of unauthorized GM events.
  It is represented in form a table, in which each row represent a GM event, where as columns represent the analytical test methods or vice versa.

- Facilitates efficient, rapid and cost-effective preliminary screening by **eliminating the need for development of specific testing methodologies for each individual GM event**.

- Regularly updated GMO databases connected to GMO matrix, currently introducing all new GM events and corresponding targets, would allow reliable and cost-efficient testing of presented GMOs globally in upcoming years.

- Multiplex PCR assays will be developed targeting *P-35S, T-nos, Os-Msca1, cry1Ab, cry1Ac, cry1C, cry2Ab, GA20 oxidase1, nptII, bar,* to facilitate preliminary screening for presence/absence of 141 GM events



Image credit: Flickr/Maria Fleischmann/World Bank

Speed read

- India's GM labelling laws cannot be enforced for lack of testing labs
- Scientists develop simple and cheap test to screen genetic contamination
- New screening matrix detects all gene components used in transgenic crops and food

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[NEW DELHI] A new algorithm for rapid screening of transgenic content could help cheaper and easier detection of genetic contamination of traditional crop varieties, food and feed, researchers say.

The algorithm, developed by scientists from India and Slovenia, is capable of detecting all 106 transgenic elements currently in use in India. Under a January 2013 law India requires labelling of genetically modified (GM) crop products, but enforcement has been hampered by a lack of testing laboratories, says Gurinder Jit Randhawa, principal scientist at the National Bureau of Plant Genetic Resources (NBPGR).

The method, developed by Randhawa and his colleagues at NBPGR, and Slovenia's National Institute of Biology, drastically cuts the number of tests required to see whether a crop, food or feed is contaminated with GM material, from over 100 to between one and ten.

The matrix-based method also cuts screening costs to one-tenth. Its design ensures that newer genes or gene pieces being tested in later tests can be added to the screening matrix.

SciDevNet
Bringing science and development together through news and analysis

Dated: 22-01-2014

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The matrix-based method also cuts screening costs to one-tenth. Its design ensures that newer genes or gene pieces being tested in later tests can be added to the screening matrix.

Instead of having to screen samples for all 106 genetic elements, the matrix uses a software 'GMOseek' to check whether a sample contains one or more foreign genes or gene pieces commonly used for genetic modification in India. These include genetic material used both in crops approved for commercial cultivation (only GM cotton so far), and in field trials or research studies a report in the April 2014 issue of journal *Food Control*, says.

Twenty one GM crops, including brinjal (eggplant), cabbage, cauliflower, chickpea, corn, cotton, groundnut, mustard, okra, papaya, potato, rapeseed, rice, rubber, sorghum, soybean, sugarcane, tobacco, tomato, watermelon and wheat are in various stages of development in India.

Success story on GMO matrix: telecasted in Gyan Vigyan (on 31 May, 2014) and Science Monitor (on 1 June, 2014) on Rajya Sabha TV (RSTV)

https://www.youtube.com/watch?v=Xo2lyZGfwuo&list=PLVOgwA_DiGzqtWKFT4KpQY32hmIy6H116

https://www.youtube.com/watch?v=u858QTdkWQk&list=PLVOgwA_DiGzpd3_Iz7J-81Vh4QqU-ZGA9

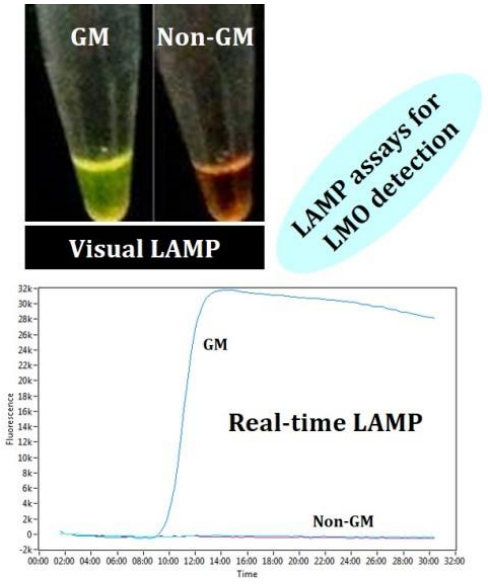
Development of Rapid and Reliable Visual and Real-time LAMP Assays for On-site GMO Screening

LAMP is an isothermal nucleic acid amplification technique, is less sensitive to inhibitors, does not require sophisticated equipment, and has the potential to be deployed on-site.

LAMP is gaining growing attention due to its high sensitivity and specificity, being superior to PCR, and usually comparable to real-time PCR. In addition, LAMP provides results on-site in a significantly shorter time as compared to conventional PCR-based techniques.

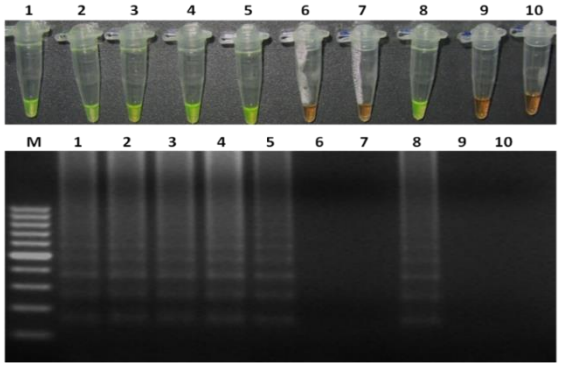
A newer generation cost-efficient GM diagnostics

- Real-time LAMP, when combined with efficient DNA extraction method, facilitates on-site GM detection in farmers' fields and at ports of entry;
- Ideal for end-point diagnostic;
- Higher sensitivity and specificity;
- User-friendly and easy to carry;
- Can be subjected to test crude DNA samples.

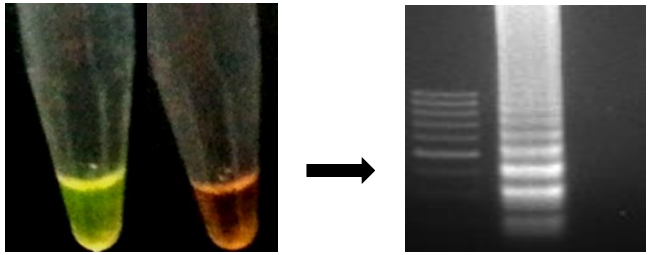


Visual LAMP

Promoters
P-35S
P-FMV
Marker genes
aadA
nptII
uidA



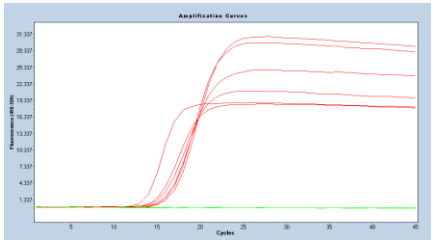
Detection of *nptII* marker gene
Green color: Positive test samples
Orange color: Negative test samples

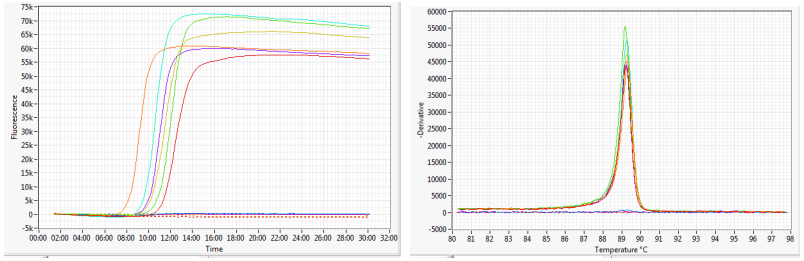


MON531 Non GM cotton **MON531 Non GM cotton**

Detection of *CaMV35S* promoter

Real-time LAMP





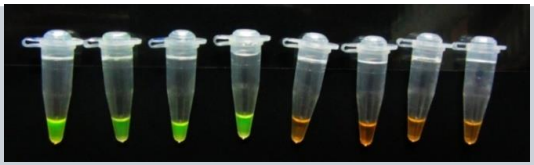
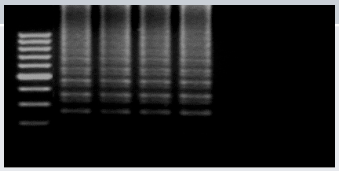
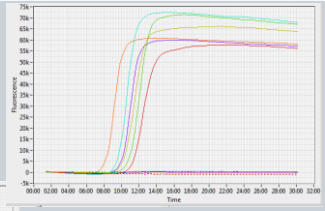
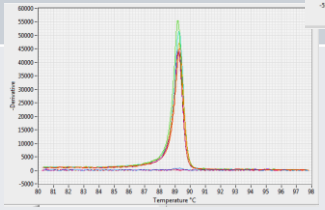
Amplification and annealing curves for LAMP assays on isothermal real-time system (Genie® II)

Amplification profile and melting curves for real-time LAMP products on Light Cycler 480 system



LAMP assays when combined with a fast DNA extraction method can be used for rapid, cost-effective and on-site detection of GM crops at port of entry and farmers' fields

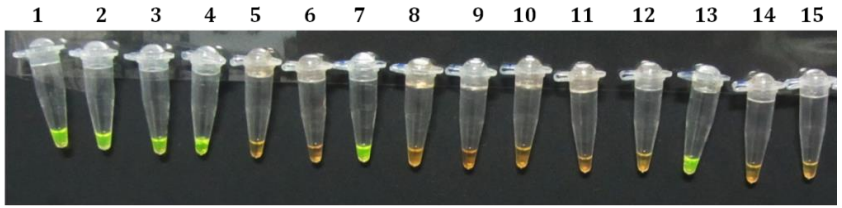
LAMP assays using two chemistries and four detection systems were compared and real-time LAMP on Genie® II was found most efficient detecting 4 copies of GM content within 35 min

System	Chemistry	Detection Method	LOD	Completion Time
Heating Block	Bst DNA Pol.		40 copies	75 min
Thermal cycler	Bst DNA Pol.	End Point 	40 copies	75 min
Light Cycler®480 Real-time System	OptiGene Isothermal Master Mix	Real-time 	10 copies	45 min
Isothermal Real-time System (Genie II)	OptiGene Isothermal Master Mix		4 copies	35 min

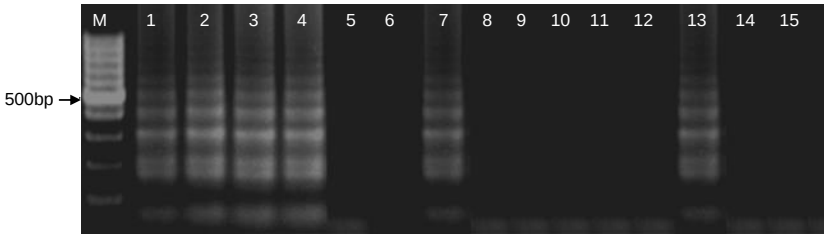
Source: Randhawa GJ, Singh M, Morisset D, Sood P and Žel J (2013) Loop-mediated isothermal amplification: Rapid, visual and real-time methods for detection of GM crops. *J. Agric. Food Chem.* , 61(47): 11338-11346.

Development of visual and real-time LAMP assays targeting commonly employed transgenes *(for screening >145 globally commercialized GM events)*

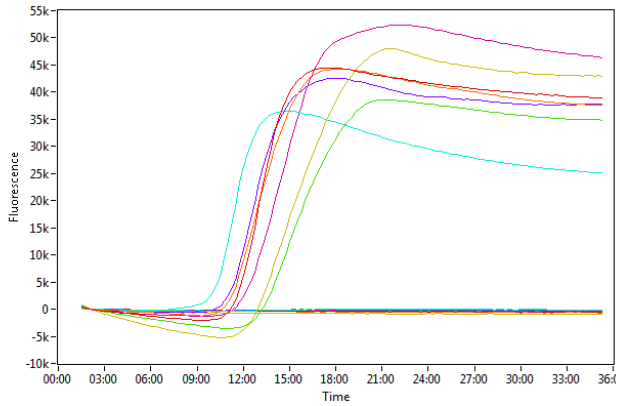
Transgenes
cry1Ac
cry2Ab2
cp4-epsps



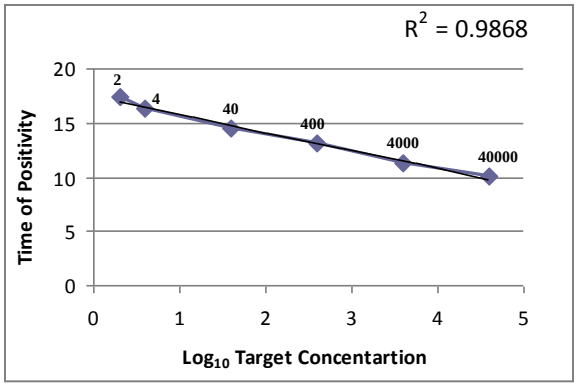
Detection of *cry1Ac* gene
Green color: Positive test samples
Orange color: Negative test samples



Specificity confirmed by ladder-like pattern of LAMP amplicons on agarose gel



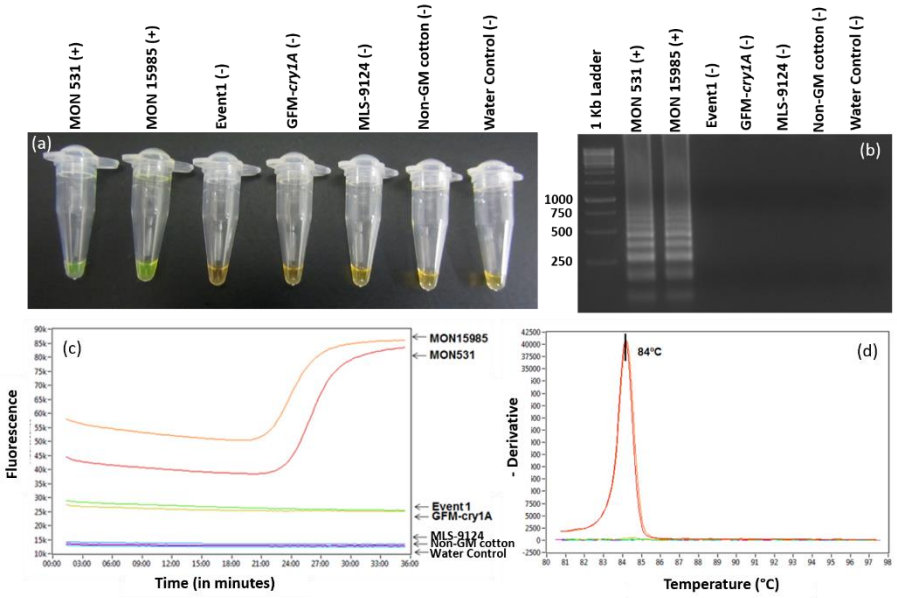
Amplification curves for LAMP assays detecting *cry1Ac* gene on isothermal real-time system (Genie® II)



Quantification limit is up to 2 copies of target (*cry1Ac*)

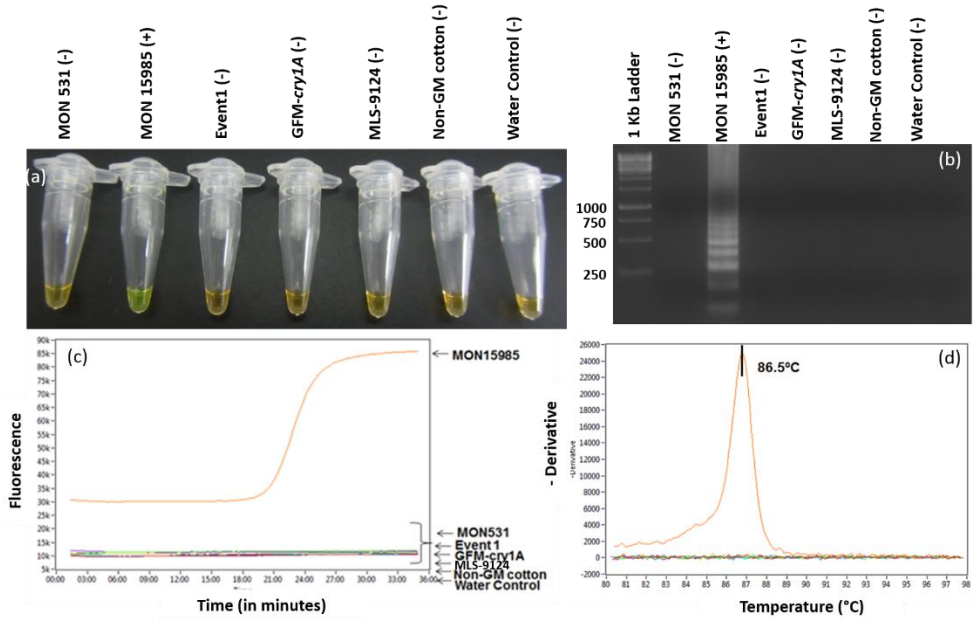
Singh M, Randhawa GJ, Sood P & Bhoge RK (2015) Loop-mediated isothermal amplification targeting insect resistant and herbicide tolerant transgenes: Monitoring for GM contamination in supply chain. *Food Cont.* 51: 283-292

Event-specific LAMP-based Diagnostics for Commercialized *Bt* Cotton Events



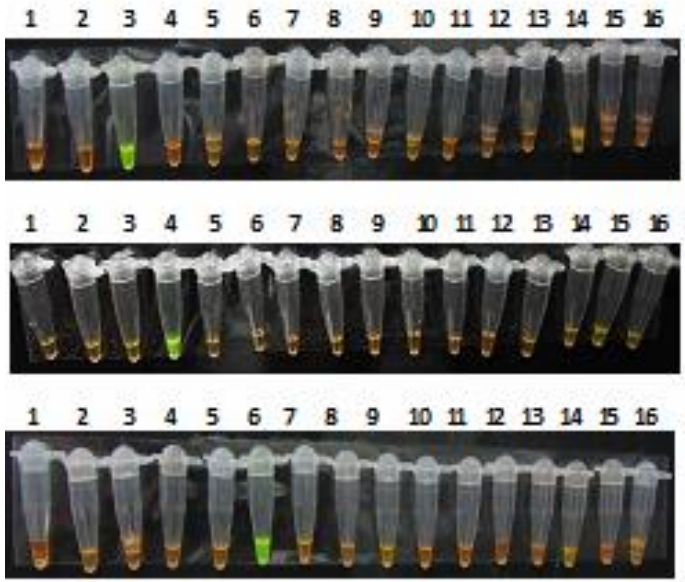
Visual and Real-time based LAMP assays for detection of *Bt* cotton event MON531 (Bollgard® I)

Visual and Real-time based LAMP assays for detection of Bt cotton event MON15985 (Bollgard® II)



Source: Randhawa et al., (2015), Visual and Real-time Event-Specific Loop-Mediated Isothermal Amplification-based Detection Assays for Bt Cotton Events MON531 and MON15985. j. AOAC Int. (In press)

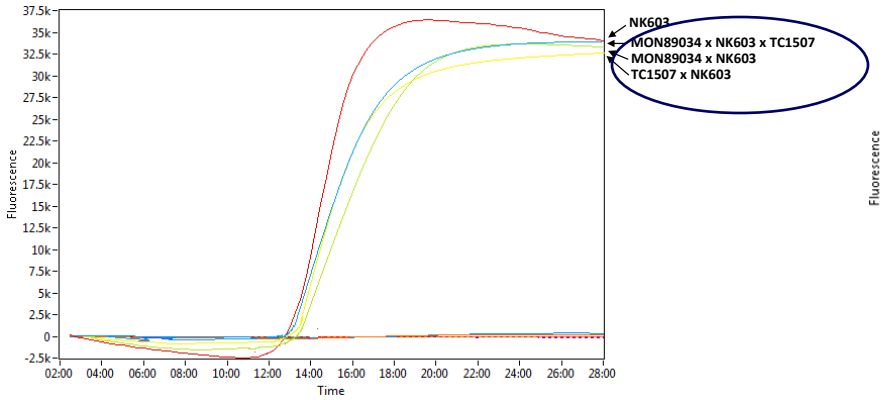
Event-specific Visual and Real-time LAMP for Six imported GM maize Events, Bt11, GA21, MON810, MON89034, NK603 and TC1507

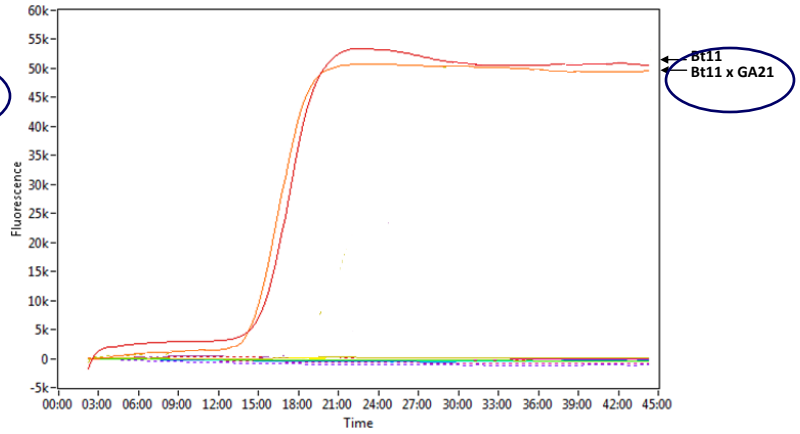


Visual LAMP for testing specificity of event-specific assays

Real-time LAMP for testing practical utility

Imported Stacked Events





Ref: RK Bhoge, R Chhabra, GJ Randhawa, M Sathiyabama & M Singh (2015) Event-specific analytical methods for six GM maize events using visual and real-time loop-mediated isothermal amplification. Food Control, doi: 10.1016/j.foodcont.2015.02.016.

Real-time PCR based Multi-target System for GMO Screening

47 Targets:

Events (21): Maize (12), Cotton (6), Rice (1), Brinjal (1), Soybean (1)
Endogenous genes (6): Maize, Cotton, Rice, Brinjal, Soybean, Potato
Construct-specific (5), Transgenes (11), Control elements (4)

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Adh1*	GA21	3272	LL Rice	*cry1Ac*	*P-35S*	*Adh1*	GA21	3272	LL Rice	*cry1Ac*	*P-35S*
B	*Sad1*	NK603	59122	EE1	*cry1Ab*	*P-nos*	*Sad1*	NK603	59122	EE1	*cry1Ab*	*P-nos*
C	*sps*	MIR162	MON531	GTS 40-3-2	*cry1Ab/Ac*	*T-nos*	*sps*	MIR162	MON531	GTS 40-3-2	*cry1Ab/Ac*	*T-nos*
D	*β-fructosidase*	MIR604	MON15985	*P35S - cry1Ac*	*cry2Ab*	*T-35S*	*β-fructosidase*	MIR604	MON15985	*P35S - cry1Ac*	*cry2Ab*	*T-35S*
E	*Lectin*	MON810	MON1445	*P35S-cry1C*	*cry3A*	*nptII*	*Lectin*	MON810	MON1445	*P35S-cry1C*	*cry3A*	*nptII*
F	*UGPase*	MON863	MON88913	*P35S-uidA*	*cry1C*	*pat*	*UGPase*	MON863	MON88913	*P35S-uidA*	*cry1C*	*pat*
G	Bt11	MON89034	Event281	*ctp2-cry2Ab*	*epsps*	*bar*	Bt11	MON89034	Event281	*ctp2-cry2Ab*	*epsps*	*bar*
H	Bt176	TC1507	Event3006	*ctp2-cp4epsps*	*AmA1*	control	Bt176	TC1507	Event3006	*ctp2-cp4epsps*	*AmA1*	control

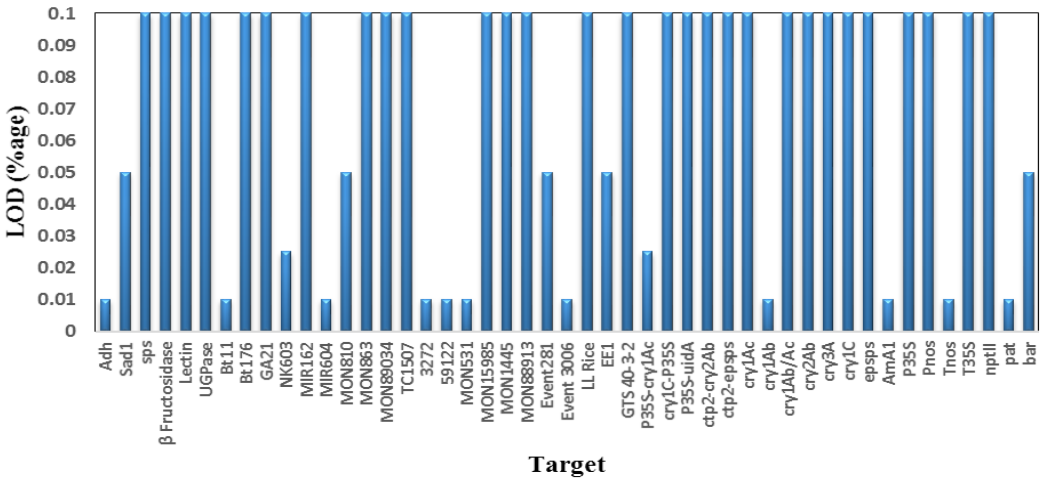
Targets for screening/ detection of commercialized *Bt* cotton events in India

Limit of Detection : Up to 0.1-0.01% for different targets

Randhawa GJ, Singh M, Sood P & Bhoge RK (2014) **Multi-target real-time PCR-based system: Monitoring for unauthorised GM events in India.** *J. Agric. Food Chem.* 62 (29), 7118 -7130.

47 Targets for ready-to-use real-time multi-target screening plate

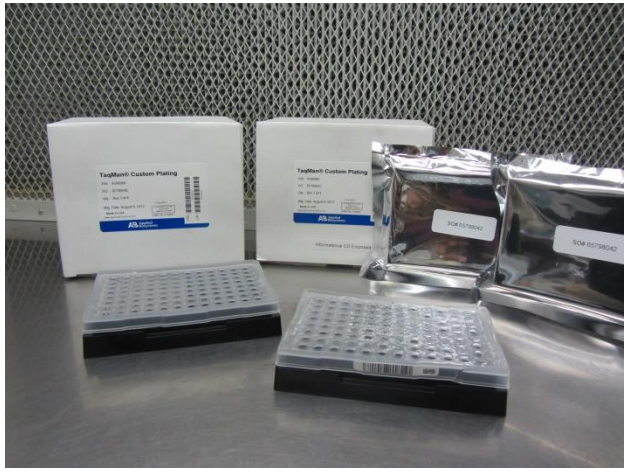
Maize	Cotton	Rice	Brinjal	Soy
Bt11	MON531	LL Rice	EE1	GTS 40-3-2
Bt176	MON15985			
GA21	MON1445			
NK603	MON88931			
MIR162	Event281			
MIR604	Event3006			
MON810				
MON863				
MON89034				
TC1507				
3272				
59122				



Endogenous Gene	Crop
Adh1	Maize
Sad1	Cotton
Sps	Rice
β-fructosidase	Brinjal
Lectin	Soy
UGPase	Potato

Limit of Detection : Upto 0.1-0.01% for different targets

Constructs	Transgenes	Promoters	Terminators	Marker genes
P-35-cry1Ac	*cry1Ac*	*P-35S*	*T-nos*	*nptII*
cry1C-P-35	*cry1Ab*	*P-nos*	*T-35S*	*pat*
P-35-uidA	*cry1Ab/Ac*			*bar*
ctp2-cry2Ab	*cry2Ab*			
ctp2-epsps	*cry1C*			
	epsps			
	AmA1			

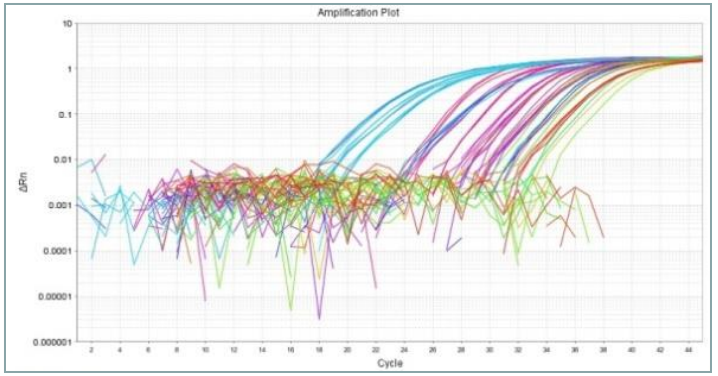


Participation in Proficiency/Comparative Testings and International Validations: Quality Assurance and Global Harmonization

- Participation in **Fifteen Real-time PCR based Comparative/Proficiency Testings** as per ISO/IEC 17043:2010 Accreditation since 2010
  - ✓ **Ten Real-time PCR based Comparative Testings organized by Joint Research Centre, Italy, European Commission (EC-JRC)**
  - ✓ **Five Real-time PCR based Comparative Testings organized by Grain Inspection, Packers & Stockyards Administration and United States Department of Agriculture (GIPSA-USDA)**

- Participated in ring trial for the validation of two real-time PCR methods, *viz.*, *cry1Ab/Ac* and *pubi-cry*, organized by **Federal Office of Consumer Protection and Food Safety, Germany in 2012**.

- Participated in Ring trial for validation of event-specific method for GM rice event Golden Rice 2 using real-time PCR organized by **EC-JRC, EURL for GM Food and Feed in 2013**



Real-time PCR profiles were in line with the expected results of ring trial

Agrinnovate India Ltd, ICAR - NBPGR and DSS Imagetech Pvt. Ltd. marks beginning of promising partnership

Agrinnovate along with NBPGR has successfully inked a deal with M/s DSS Imagetech Pvt. Ltd. on 19th August, 2015 for transfer of DNA-based GMO screening technologies





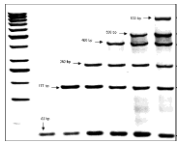
Dr. J. S. Sandhu, Dr. K. C. Bansal, S. Ravinderjit Singh, Dr. Ajay Kandhari, Director, DSS Imagetech
Technology Developers: Dr. Gurinderjit Randhawa & Dr. Monika Singh
Team Members of AgIn & DSS Imagetech at MoA Signing Ceremony

The technology and knowledge sharing is a step towards national capacity building and make in India effort.

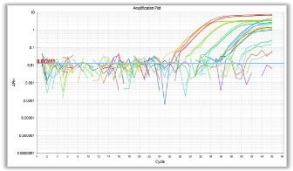
Five DNA-based GMO Screening Technologies Transferred

GMO Screening Technologies

1. Hexaplex PCR simultaneously targeting commonly employed six marker genes, namely, *aadA, bar, pat, nptII, hpt, uidA*

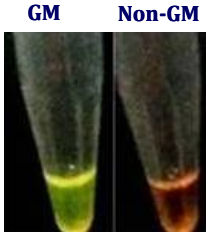


2. Duplex TaqMan® Real-time PCR simultaneously targeting *CaMV* 35S promoter and *nos* terminator

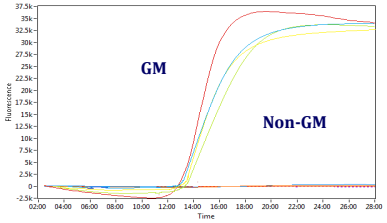


Can detect more than 90% of globally commercialized GM events, including all commercialized GM cotton events in India

3. Visual Loop-mediated Isothermal Amplification (LAMP) targeting eight transgenic elements (*P-35S, T-nos, aadA, nptII, uidA, cry1Ac, cry2Ab, cp4-epsps*), positive tests can be visually detected by colorimetric change

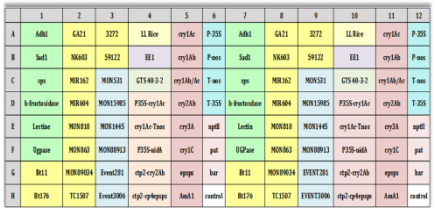


4. Real-time LAMP targeting eight transgenic elements (*P-35S, T-nos, aadA, nptII, uidA, cry1Ac, cry2Ab, cp4-epsps*)



*Can screen more than 80% of globally commercialized GM events, including all commercialized GM cotton events in India, **facilitating rapid/cost-efficient on-site GMO screening***

5. TaqMan Real-time PCR based Multi-target Technology detecting 47 targets would assist in *rapid GMO screening for a wide range of GM crops/events* including cotton, maize, rice, brinjal, potato and soybean



Public-Private Partnership for PCR-based GMO detection: Technology Transfer





MoU was signed between NBPGR and Punjab Biotechnology Incubator (PBTI), Mohali (Dr. R. S. Marwaha, CEO, PBTI) for transferring PCR-based GM detection technologies/kits developed by NBPGR, New Delhi, on non-exclusive basis, in the presence of Dr. Swapan K. Datta, DDG (Crop Sciences), Dr. V. L. Chopra, Former Secretary, DARE & DG, ICAR; Former Member Secretary, Planning Commission and Dr. J. S. Chauhan, ADG (Seeds), in the 38th Foundation Day of NBPGR on 1st August, 2014

Public-Private Partnership for PCR-based GMO detection: Technology Transfer



MoU of technology transfer for GMO Detection in rice and other GM crops were exchanged in the presence of Prof. Kasturinandan, with Basmati Export Development Foundation, APEDA, in **Agri-Tech Investor Meet** at NASC Complex New Delhi on 19th July 2013



MoU was signed between NBPGR and Amar Immunodiagnostics Pvt. Ltd. Hyderabad, for transferring PCR based technologies/kits developed by NBPGR, New Delhi, in May 2010

Workshop on "Biosafety and Detection of GM Crops"

held from 11th to 16th August, 2014 at NBPGR





Shri Hem Pande, Additional Secretary, MoEF & Chairman, GEAC inaugurated the Workshop





Scientists and researchers from ICAR institutes & SAUs from all over the country participated in the Workshop.



Padma Bhushan Dr. R. S. Paroda: Chief Guest at Valedictory Session

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

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

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



Training courses at national and international levels were organized in the area of LMO/GMO detection and biosafety issues of transgenics.

More than 300 researchers from National Agricultural Research System (NARS) and other public and private organizations were trained in DNA-based diagnostics and various biosafety issues.

NAIP sponsored training programme

Molecular Diagnostics for Risk Assessment and Management of Genetically Modified Crops

(from 8th to 21st November 2011 at NBPGR, New Delhi)



Dr. J.S. Sandhu ADG (Seeds), and Dr. Karim Maredia, Professor, Michigan State University during inaugral function



Prof. S. K. Datta, DDG (Crop Science), during valedictory function



Dr. Dany Morisset and Dr. David Dobnik, Slovenian Guest Faculty

Capacity building for addressing issues related to Biosafety & GM detection

Eight Orientation Courses on Biosafety Considerations for Evaluation of Transgenic Crops have been conducted at NBPGR

(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





Brainstorming Session on 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



54 panelists/researchers participated

- **public sector: 38**
- **private sector:16**



Stocktaking Assessment of NBPGR for Implementation of UNEP/GEF supported Phase-II Capacity Building Project on Biosafety for LMO Detection in India

Visit of International Consultant Dr. Patrik Stolt (Scan Bi-Diagnostics, Sweden) for Stocktaking Assessment in GM Detection Laboratory at NBPGR (March 11-12, 2014)

Dr Stolt interacted with the team members and reviewed the facilities:

- Establishment, security, equipments and laboratories;
- Expertise and capabilities of staff;
- Data handling, storage and security;
- Maintenance of log books and laboratory records, quality control system;
- LMO detection methodologies, handling of LMOs and non-LMOs, interpretation of results;
- Participation in national/ international validations and ring trials.









As an Outcome of GM Detection Work Done.....

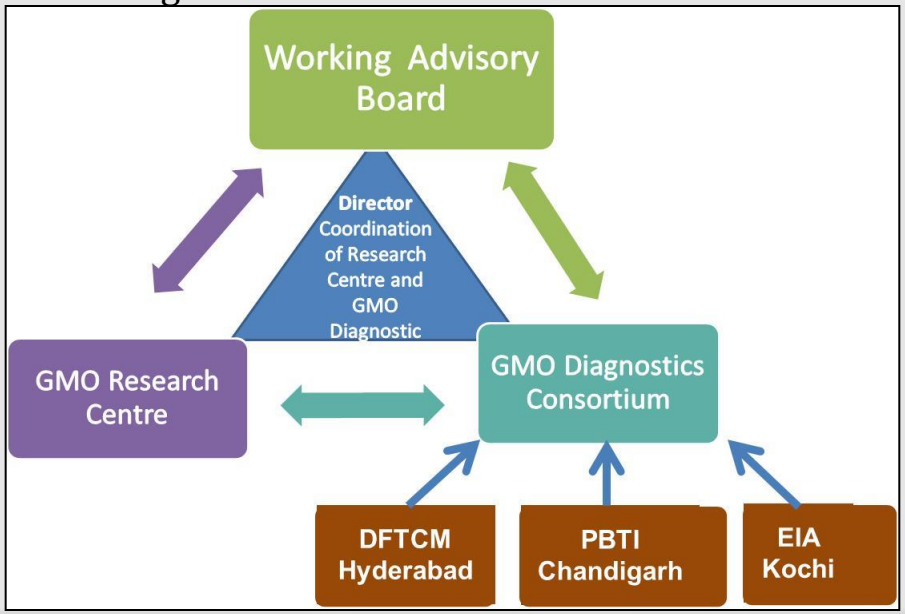
Stocktaking Assessment under GRF-UNEP Phase II Project on Biosafety:
By International Consultant Dr. Patrik Stolt, Sweden of GM Detection Laboratory
(March 11-12, 2014)



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- Establishment, security, equipments and laboratories;
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- Data handling, storage and security;
- Maintenance of log books and laboratory records, quality control system;
- LMO detection methodologies, handling of LMOs and non-LMOs, interpretation of results;
- Participation in national/ international validations and ring trials.

GM Detection Lab designated as "GMO Detection Research Centre" (GDRC) under GRF-UNEP Phase II Project on Biosafety coordinated by Ministry of Environment and Forests & Climate Change, Govt. of India



Challenges Ahead

New GMOs with more genes and more traits

Monsanto's New Leaf Plus® potato variety, which contains a total of eleven different foreign genetic elements- Resistant to Colorado Potato Beetle And Leaf Roll Virus

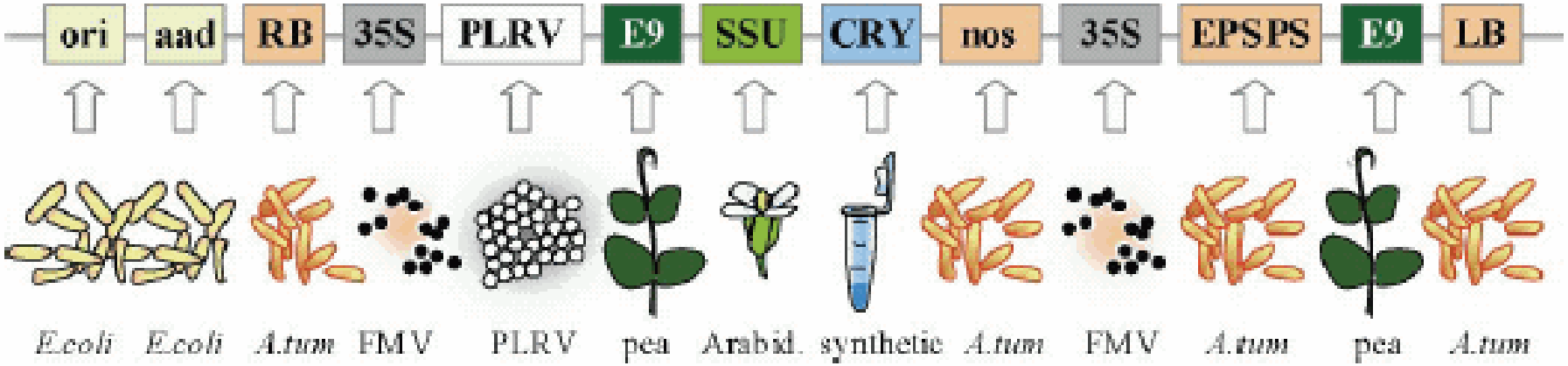


Figure 1. Schematic structure of an insertion site of NewLeaf Plus®. ori = origin of replication of *E. coli* plasmid pBR322, aad = spectinomycin resistance gene from *E. coli*; RB = right border region of *Agrobacterium tumefaciens* (*A. tum*); 35S = promoter of figwort mosaic virus; PLRV = replicase gene of potato leaf roll virus; E9 = terminator of pea E9 gene; SSU = promoter of Arabidopsis rubisco small subunit gene; CRY = synthetic gene encoding a protein identical to the cry3Aa protein of *Bacillus thuringiensis*; nos = terminator of *Agrobacterium* nopaline synthase gene; EPSPS = 5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium*; LB = left border region of *Agrobacterium*.

http://www.isb.vt.edu/articles/dec0405.htm


Thank you