Biosafety Issues and Bt cotton – A case study

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Introduction

• Classical plant breeding is limited to the introduction of required characters into plant by genetic crossing during sexual reproduction

• To feed the ever increasing population more and more food has to be produced from less and less land water and other natural resources

• It is obvious that emphasis has to be laid on new technologies that can improve crop yield against adverse conditions of biotic and abiotic stresses and improve the quality

contd…. 
• The earlier part of plant biotechnology era has been the development of tissue culture protocols of several commercially important crops.

• The “gene revolution” as compared to green revolution is poised to benefit both poor and rich farmers equally and has an immense potential in transforming global agriculture.

• In India, significant efforts have been made to significantly for developing several programmes in Biotechnology.
WHAT IS A TRANSGENIC CROP

–A transgenic crop is a crop which contains a gene or genes of a different species artificially inserted in its genome, which may come from an unrelated plant or from a completed different species.
Why make transgenic crops?

• Due to limitations of conventional breeding for attaining the desirable traits use of recombinant DNA technology has been taken advantage of and development of transgenics started
CELL: SMALLEST UNIT OF LIFE
NUCLEUS: BRAIN OF THE CELL

CHROMOSOMES: STRUCTURE CONTAINING THE GENETIC INFORMATION

GENES: SMALLER PIECES OF GENETIC INFORMATION

DNA: BUILDING BLOCK OF THE GENE, DOUBLE HELIX STRUCTURE

Organization of DNA in the cell
Biotechnology is an Extension of Traditional Plant Breeding

TRADITIONAL PLANT BREEDING

DNA is a strand of genes, much like a strand of pearls. Traditional plant breeding combines many genes at once.

PLANT BIOTECHNOLOGY

Using plant biotechnology, you can add a single gene to the strand.
Development of GM Crop/Trasgenics

- Identify gene(s) giving a desired trait
- Make copies of the gene
- Transfer to plant tissue
- Regenerate plants
- Lab analysis and safety testing
- Development of a variety
- Field tests
- Approval by Government agencies
- Commercialization
- Monitoring of efficacy and safety

7-8 years
Methods of producing transgenic plant

A. Agrobacterium
   - Bacterium mixed with plant cells
   - Ti plasmid moves into plant cell and inserts DNA into plant chromosome

B. Gene Gun
   - Gene replication
   - Gold particles coated with DNA
   - Cells shot with gene gun and DNA incorporated into plant cell chromosome

C. Screening for cells with transgene
   - Cells screened for transgene
   - Transformed cells selected with selectable marker
   - Transgenic plant regenerated from single transformed cell
### Advantages of transgenic crops over breeding

<table>
<thead>
<tr>
<th>Breeding</th>
<th>Transgenic crops</th>
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</thead>
<tbody>
<tr>
<td>Exchange of genes within a species</td>
<td>No barrier</td>
</tr>
<tr>
<td>Gene of interest with flanking sequence transferred</td>
<td>Only Gene of Interest</td>
</tr>
<tr>
<td>Simple Technology</td>
<td>Intensive Technology</td>
</tr>
<tr>
<td>History of Safety</td>
<td>New Technology Time needed for Safety History</td>
</tr>
</tbody>
</table>
Transgenic Crops: Development Objectives

- Integrated pest management
- Herbicide tolerance
- Nutritional enhancements
- Product quality improvement
- Increase in yield
- Stress tolerance
- Plant based pharmaceuticals
GLOBAL SCENARIO 2005

- First crop introduced was Flavr Savr tomato in USA in 1995
- So far 19 crops approved for commercial cultivation
- Include canola, carnation, chicory, cotton, linseed, green pepper, maize, melon, potato, rice, soybean, squash, sugarbeet, sunflower, tobacco and tomato
- Major characteristics are insect resistance, herbicide tolerance, virus resistance and improved product quality
GLOBAL SCENARIO 2005

• Major countries include USA, Canada, Japan, China, EU, Argentina, South Africa
• Only four crops being marketed commercially I.e., corn, cotton, soybean and canola
• Commercial production initiated for papaya, squash and tobacco in USA
• Others are approved but not yet being marketed
GLOBAL SCENARIO 2005

• Area under cultivation: 90 million hectares
• No. of countries: 21
• No. of farmers: 8.25 million
• Crops: Corn, soybean, cotton and canola
• Transgenic traits: insect resistance & herbicide tolerance
Global area of transgenic crops from 1996 to 2005 (million hectares)

- 1996: 1.7
- 1997: 11.0
- 1998: 27.8
- 1999: 39.9
- 2000: 44.2
- 2001: 52.6
- 2002: 58.7
- 2003: 67.7
- 2004: 81.0
- 2005: 90.0
GLOBAL AREA OF BIOTECH CROPS
Million Hectares (1996 to 2005)

Increase of 11%, 9.0 million hectares or 22 million acres, between 2004 and 2005.

Source: Clive James, 2005.
21 Biotech Crop Countries and Mega-Countries*, 2005

- **#14 Spain**
  - 0.1 Million Has.
  - Maize

- **#20 France**
  - <0.05 Million Has.
  - Maize

- **#19 Germany**
  - <0.05 Million Has.
  - Maize

- **#21 Czech Republic**
  - <0.05 Million Has.
  - Maize

- **#12 Romania**
  - 0.1 Million Has.
  - Soybean

- **#16 Iran**
  - <0.05 Million Has.
  - Rice

- **#7 India**
  - 1.3 Million Has.
  - Cotton

- **#6 China**
  - 3.3 Million Has.
  - Soybean

- **#13 Philippines**
  - 0.1 Million Has.
  - Maize

- **#10 Australia**
  - 0.3 Million Has.
  - Cotton

- **#3 Brazil**
  - 9.4 Million Has.
  - Soybean

- **#8 South Africa**
  - 0.5 Million Has.
  - Maize, Soybean, Cotton

- **#16 Mexico**
  - 0.1 Million Has.
  - Cotton, Soybean

- **#15 Colombia**
  - <0.05 Million Has.
  - Cotton

- **#10 Argentina**
  - 17.1 Million Has.
  - Maize, Cotton

- **#9 Uruguay**
  - 6.3 Million Has.
  - Soybean, Maize

- **#6 Paraguay**
  - 1.0 Million Has.
  - Soybean

* 14 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

Source: Clive James, 2005
RESEARCH & DEVELOPMENT STATUS

- 63 countries involved at various stages
- 57 plants identified for development
- Second generation of traits include modifications such as nutritional enhancement, disease tolerance, stress tolerance and production of pharmaceuticals
# Wide range of crops

<table>
<thead>
<tr>
<th>16 Field Crops</th>
<th>14 Vegetables</th>
<th>16 Fruits</th>
<th>11 other crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>Broccoli</td>
<td>Apple</td>
<td>Chicory</td>
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<tr>
<td>Barley</td>
<td>Cabbage</td>
<td>Banana</td>
<td>Cocoa</td>
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<tr>
<td>Canola</td>
<td>Carrot</td>
<td>Cantaloupe</td>
<td>Coffee</td>
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<tr>
<td>Cassava</td>
<td>Cauliflower</td>
<td>Cherry</td>
<td>Garlic</td>
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<tr>
<td>Clover</td>
<td>Cucumber</td>
<td>Citrus</td>
<td>Lupins</td>
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<tr>
<td>Cotton</td>
<td>Eggplant</td>
<td>Coconut</td>
<td>Mustard</td>
</tr>
<tr>
<td>Flax</td>
<td>Lettuce</td>
<td>Grape</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>Maize</td>
<td>Onion</td>
<td>Kiwi</td>
<td>Oilseed Poppy</td>
</tr>
<tr>
<td>Rice</td>
<td>Pea/Bean</td>
<td>Mango</td>
<td>Olive</td>
</tr>
<tr>
<td>Safflower</td>
<td>Pepper</td>
<td>Melon</td>
<td>Peanut</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Potato</td>
<td>Papaya</td>
<td>Tobacco</td>
</tr>
<tr>
<td>Soybean</td>
<td>Spinach</td>
<td>Pineapple</td>
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</tr>
<tr>
<td>Sugar Beet</td>
<td>Squash</td>
<td>Plum</td>
<td></td>
</tr>
<tr>
<td>Sugar Cane</td>
<td>Tomato</td>
<td>Raspberry</td>
<td></td>
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<tr>
<td>Sunflower</td>
<td></td>
<td>Strawberry</td>
<td></td>
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<tr>
<td>Wheat</td>
<td></td>
<td>Watermelon</td>
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COMMERCIAL USE IN INDIA

- Only one crop approved i.e. Bt cotton
- Three hybrids containing Cry1Ac gene approved in 2002 and one in 2004
- 62 hybrids approved for Kharif 2006
- Three new events approved i.e.
  i. Cry 1Ac gene (event 1) by M/s J.K. Agri Seeds Ltd.
  ii. Fusion genes (cry 1Ab+cry 1Ac) 'GFM' by M/s Nath Seeds
  iii. Stacked genes cry1Ac and cry1Ab by M/s Mahyco
PRE-Bt COTTON PRODUCTION & Bt DEVELOPMENT

- Area, Production, Productivity of the decade prior to Bt introduction
- Productivity of Rain-fed vs Irrigated Cotton pre-Bt period
- Indian Cotton Vs International Cotton
- Sequence of Development of Bt Cotton
- Economics of first generation Bt Cotton in country-wide coordinated trials
## Bt Cotton Development Process in India

### Environment Safety
- Pollen flow: 1997
- Aggressiveness: 97-98
- Pollen flow (R): 2001
- Soil/organism: 2001-02
- Gene stability: 2001

### Bio-efficacy & Agronomic Benefit
- Field Trials (nos.):
  - 98-99: 40
  - 99-0: 19
  - 00-01: 25
  - 01-02: 376

### Biosafety Studies
- Allergenicity: 98
- Goat: 98
- Cow, Buffalo, Chicken: 2001
- Fish Studies: 2001
- Protein: 2001
- Toxin: 2001

### GEAC Data Evaluation 2001

### Seed Bulk Approved 2001

### Commercialization 5th April 2002
BIOSAFETY ASSESSMENT

- STUDIES ON ENVIRONMENTAL SAFETY
- STUDIES ON FOOD SAFETY
- RISK ASSESSMENT
1. STUDIES ON ENVIRONMENTAL SAFETY

POLLEN ESCAPE/OUT CROSSING:

- Due to stickiness pollen travel is limited
- No chance of transfer from tetraploid Bt hybrids to cultivated diploid species
- No wild species occur in India
AGGRESSIVENESS AND WEEDINESS

TO ASSESS THE WEEDINESS OF BT COTTON, THE RATE OF GERMINATION AND VIGOUR WERE COMPARED WITH NON BT COUNTERPARTS

- No significant differences between Bt and non Bt cotton for germination and vigor
- Hence there is no difference between Bt and non Bt cotton with regard to their weediness and aggressiveness potential
EFFECT OF Bt ON NON TARGET ORGANISMS

STUDIES CONDUCTED DURING THE MULTI LOCATION FIELD TRIALS

- Bt cotton hybrids did not have any TOXIC effects on non target species such as:
  - Sucking pest (aphids, jassids, whitefly and mites)
  - The beneficial insects (ladybird, beetle, honeybees and spiders) remained active in both Bt and Non Bt cotton hybrids.
PRESENCE OF Bt PROTEIN IN SOIL

STUDIES WERE CONDUCTED TO ASSESS THE POSSIBLE RISK OF ACCUMULATION OF BT PROTEIN IN THE SOIL

- Bt protein was not detected in soil samples indicating that Cry1Ac protein was rapidly degraded in the soil.
- The half-life of the Cry1Ac protein in plant tissues was calculated to be 41 days which is comparable to the degradation rates reported for microbial formulations of Bt.
EFFECT OF BT PROTEIN ON SOIL MICROFLORA

STUDIES WERE CONDUCTED TO EVALUATE ANY IMPACT OF BT PROTEIN LEACHED BY ROOTS OF BT COTTON ON THE SOIL MICROFLORA

- Results showed that there was no significant difference in population of microbes and soil invertebrates (like earthworms) between Bt and non Bt samples
2. STUDIES ON FOOD SAFETY

FOR EVALUATING FOOD SAFETY, THE STUDIES INCLUDED:

- Compositional analysis
- Allergenicity studies
- Toxicological studies
- Presence of Bt protein in cotton seed oil
- Feeding studies on cows, buffaloes, poultry and fish.
COMPOSITIONAL ANALYSIS

STUDIES REVEALED THAT THERE WAS NO CHANGE IN THE COMPOSITION IN THE Bt AND NON Bt COTTON SEEDS WITH RESPECT TO:

- Proteins
- Carbohydrates
- Oil
- Calories
- Ash content
ALLERGENICITY STUDIES

ALLERGENICITY STUDIES WERE CONDUCTED ON BROWN NORWAY RATS BY FEEDING Bt AND NON Bt SEEDS. RESULTS SHOWED THAT:

- No significant difference in feed consumption, weight gain and general health
- There was no significant change in endogenous allergens of Bt seed compared to non Bt cotton seed
GOAT FEEDING STUDY WAS CONDUCTED FOR UNDERSTANDING THE TOXICOLOGICAL EFFECTS OF Bt COTTON SEED. THE ANIMALS WERE ASSESSED FOR GROSS PATHOLOGY AND HISTOPATHOLOGY:

- No significant differences were found between animals fed with Bt and non Bt cotton seed
PRESENCE OF Cry1Ac PROTEIN IN Bt COTTON SEED OIL

STUDIES HAVE INDICATED THAT Cry1Ac PROTEIN WAS NOT FOUND IN REFINED OIL OBTAINED FROM Bt COTTON SEEDS
FEEDING STUDIES ON COWS, BUFFALOES, POULTRY AND FISH

- The feeding experiments using Bt cotton seed meal were conducted at National Dairy Research Institute, Karnal on lactating cows;
- Department of Animal Nutrition, College of Veterinary Sciences, G.B. Pant University of Agriculture & Technology, Panchnagar on lactating buffaloes.
- Central Avian Research Institute, Izatnagar on poultry
- Central Institute on Fisheries Education, Mumbai on fish

These experiments indicated that Bt cotton seed meal was nutritionally as wholesome and safe as the non Bt cotton seed meal.
3. RISK MANAGEMENT

PEST POPULATION EXPOSED TO Bt CROPS CONTINUOUSLY FOR SEVERAL YEARS MAY DEVELOP RESISTANCE TO THE Bt TOXINS THROUGH NATURAL SELECTION MUTATION, AND SELECTION:

- To prevent resistance build up it is recommended to plant sufficient non Bt cotton (20%) to serve as a refuge for Bt susceptibility in seeds.
- The refuge strategy is designed to ensure that Bt susceptible insects will be available to mate with Bt resistant insects, should they arise.
- Available genetic data indicates that susceptibility is dominant over resistance.
- Therefore, the offspring of these matings would most likely be Bt susceptible, thus mitigating the spread of resistance in the populations.
4. OTHERS SAFEGUARDS

CONFIRMATION OF THE ABSENCE OF “TERMINATOR TECHNOLOGY”

- Molecular detection test in the Bt cotton hybrids was performed for absence of any terminator gene.
5. CONDITIONS STIPULATED BY GEAC

- Valid for three years: April 02 to March, 05
- Three hybrids namely MECH12Bt, MECH162Bt and MECH184Bt
- Provide same non Bt seed to meet refuge requirements
- Conduct studies to monitor resistance development
- Provide information to government on distribution of the seed through its dealers and agents
- Labeling requirements such as GEAC number, etc.,
- Develop Bt based IPM program
- Undertake education and awareness program
- Meet other requirements as stipulated
## State-wise Adoption of Bt-Cotton (000’ha)

<table>
<thead>
<tr>
<th>State</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>% Growth</th>
<th>% area covered in 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andhra Pradesh</td>
<td>3.8</td>
<td>5.2</td>
<td>74.9</td>
<td>226.7</td>
<td>597</td>
<td>22.9</td>
</tr>
<tr>
<td>Karnataka</td>
<td>2.2</td>
<td>3.6</td>
<td>20.5</td>
<td>28.9</td>
<td>1214</td>
<td>08.0</td>
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<tr>
<td>Tamil Nadu</td>
<td>0.4</td>
<td>3.4</td>
<td>9.8</td>
<td>18.4</td>
<td>4500</td>
<td>07.8</td>
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<tr>
<td>Maharashtra</td>
<td>12.4</td>
<td>18.7</td>
<td>208.7</td>
<td>621.1</td>
<td>4909</td>
<td>20.9</td>
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<tr>
<td>Gujarat*</td>
<td>9.1</td>
<td>42.1</td>
<td>134.0</td>
<td>147.4</td>
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<td>07.2</td>
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<td>87.9</td>
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<td>Punjab</td>
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<td>1.6</td>
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Area under Bt cotton cultivation in India
Thanks Thanks