Environmental Risk Assessment and Management of Living Modified Organisms (LMO)

Hyen-Mi CHUNG, Director
Environmental Biosafety Division, NIER
National Institute of Environmental Research
Contents

1. Background: Biotechnology and LMO Risk Management
2. Role of NIER in Korean System
3. Environmental Risk Assessment research
Biotechnology & LMO

1953

• DNA ds Structure (1953)

1960

• Green Revolution

1970

• 1st Lab test with LM animal; LM rabbit embryo (1971)

• Construction of recombinant DNA (1972)

• Gene modified E. coli (1973)

• Dev. Of Insulin producing LM MO (1977)

1980

• 1st Vaccine production from LMO (Virbad SA, 1988)

• 1st LM animal patent - oncomouse (1984)

• Microinjection Tech. (1980)

• Approval of Insulin prod. from LM MO (1982)

• LM plant (1983 Petunia)

• Human growth hormone (1986), antibody (1989) from LM tobacco

1990

• Cloned sheep Dolly (1997)

• Lactoferrin milk cow, ‘Herman’ (1990)

• Flavr Savr Tomato (1994)

• Commercial LM crops (1996)

• HBV vaccine from GM tobacco (1992)

2000

• Recombinant human antithrombin from GM goat, (ATryn, 2006)

• Golden rice (2000)

• Blue rose (2004)

• LM crops production (114.3 mha, 2007)

• Swine cloning for organ transplant (2002)

향후

• Stress resistant Crops

• Bioenergy, Medical related R&D

National Institute of Environmental Research
National Institute of Environmental Research

GM CROPS Worldwide

Biotech Crop Countries and Mega-Countries*, 2007

1. Portugal 0.05 Million Has. Maize
2. Canada 0.05 Million Has. Maize, Soybean
3. USA 0.1 Million Has. Maize, Soybean, Maize, Cotton, Canola, Squash, Pumpkins, Alfalfa
4. Mexico 0.1 Million Has. Cotton, Soybean
5. Honduras 0.05 Million Has. Maize
6. Colombia 0.05 Million Has. Cotton, Canola
7. Argentina 0.1 Million Has. Maize, Soybean, Carolina
8. Uruguay 0.2 Million Has. Soybean, Maize
9. Paraguay 0.2 Million Has. Soybean
10. Brazil 0.4 Million Has. Soybean, Maize
11. South Africa 0.1 Million Has. Maize, Soybean, Cotton
12. Spain 0.1 Million Has. Maize
13. France 0.05 Million Has. Maize
14. Germany 0.05 Million Has. Maize
15. Czech Republic 0.05 Million Has. Maize
16. Poland 0.05 Million Has. Maize
17. Slovakia 0.05 Million Has. Maize
18. Romania 0.05 Million Has. Maize
19. China 0.8 Million Has. Cotton, Tomato, Potato, Peanut, Papaya, Sweet Pepper
20. India 6.2 Million Has. Cotton
21. Philippines 0.3 Million Has. Maize
22. Australia 0.1 Million Has. Cotton

* 13 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.
Source: Clive James, 2007.

GM crops in 114.3 million ha, 23 countries in the world

Crops [2007]

<table>
<thead>
<tr>
<th>Country</th>
<th>Soybean</th>
<th>Maize</th>
<th>Cotton</th>
<th>Canola</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Traits (2007)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Herbicide R</th>
<th>Insect R</th>
<th>Combinant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>72.2</td>
<td>20.3</td>
<td>21.8</td>
</tr>
</tbody>
</table>

National Institute of Environmental Research
Potential Adverse Effects on LMO

Human Health

- **Direct effects** of inserted gene and gene products:
  - Toxicity, allergy, antibiotic resistance induction
- **Indirect effects** of inserted gene and gene products:
  - Unintended formation of materials and function from the nutrients or intermediate products of metabolisms
- **Gene transfer** to the MO’s in the digestive tract

Environment

- **Gene transfer**: to the related wild life (center of origin)
- **Weedness**: Survival in the wild environment
- Adverse effect on the biological diversity
- Effects on the non-target organisms: animal, insects etc.

Technology and Market

- **Profit from exclusive LMO technology**
- **Patent on LMO seeds**
- **Distribution network**

Examples:

- **Brazil nut (1996)**
  - Invalid commercialization due to allergen induction
- **GM Potato (Pusztai, 1998)**
  - Potential risk dispute on the growth and immune system on the mouse
- **Monarch butterfly (1999-2001)**
  - Larvae death from GM pollen (Bt maize)
    - GM canola growth beside the road from transportation
- **StarLink corn (2000)**
  - Corns approved for feed introduced to the ones for food network
- **LLRice 601 (2006)**
  - Unintended mixing of rice not approved
Role of NIER in Korean System
1. Korean Law: The Transboundary Movement of Living Modified Organisms (No.6446, so called LMO’s Act)

- **2000. 1** Adoption of Cartagena Protocol on Biosafety to the Convention on Biological Diversity
- **2000. 9** Signed the Protocol
- **2001. 3** Adoption of LMO’s Act
- **2003. 9** International Effectuation of Biosafety Protocol
- **2005-6** The Enforcement Ordinance of the Act
- **2007** The corporate notification of the Act
- **2008. 1** Ratification of Biosafety Protocol and Effectuation of LMO’s Act
Role of Ministry of Environment

- **Management of the risk of LMOs, the potential adverse effects on the conservation and sustainable use of biological diversity**
  - Consulting on the ERA of LMO for approval

- **Management for development, production, import, export, transporting and storage of LMO for bioremediation**
  - Bioremediation can be defined as any process that uses LMO (microorganisms, fungi, green plants) to return the natural environment altered by contaminants to its original condition
  - ex) Plant a heavy metal resistance poplar on closed mine or factory

※ Based on
- **The Cartagena Protocol on Biosafety** (Article 15, 16)
- **LMO’s Act: Trans-boundary movement of LMO** (Article 7, 13)
- **Wild Animal and Plant Protection Law** (Article 2, 25)
  - LMO has been assigned as “ecosystem disturbance organisms”
  - Responsibility on investigation and management of LMO
Mission NIER on Biosafety Management

- **Evaluation**
  - Environmental risk evaluation for import/production approval on LMO for bioremediation
  - Consulting on environment risk

- **Assessment**
  - Research on domestic environmental risk assessment of LMO

- **Management**
  - Management and post-marketing monitoring of deliberately released LMO in domestic ecosystem
  - Management of domestic distribution, production and marketing process of LMO for bioremediation

- **Public & Int’l Cooperation**
  - OECD working party on biotechnology for harmonization of RA methodology
  - Information management by LESC for public relations

---

National Institute of Environmental Research
Studies for Environmental Risk Assessment (ERA) of LMO
Model Studies of LMO for ERA

- Model study of gene transfer of GM microorganisms in soil environment (*Alcaligenes* sp. JMP228/pJP4, *Burkholderia cepacia*(kanamycin*, bacitracin*))

- Fate of GM *Escherichia coli* and engineered DNA in natural soils by unintentional release (*E. coli* HB101/pJP4)

- ERA of Model Transgenic weed for potential use of bioremediation

- ERA of virus resistant transgenic tobacco plants
BACKGROUNDS
Practicing the Whole ERA Procedure

Construction of model Transgenic plant
- w/ mer genes, marker
- Agrobacterium method
- Parental organisms, donor organisms, Vector

LMO identity
- Confirmation of inserted gene and traits (mercury resist)
- Gene stability: ratio of separation; exp. of the traits in generations
- Biological comparability: morphology, physiology, growth charac.

Effects on the Receiving Env.
- Effects on the Community: rhizosphere MOs, insects etc
- Gene transfer: target & non-target: relative sp. MOs, insects, etc
**Function of Mercury Resistance Gene**

- MeHg\(^+\) (organic mercury) → \textit{merB} → Hg\(^{2+}\) (ionic mercury) → \textit{merA} → Hg\(^0\) (elemental mercury)

**Parental Plant, Introduced Genes, Donor organisms & Vector**

- **Solanum nigrum** L.
  - Black nightshade, Common nightshade
  - Seed breeding, annual weeds

- **merB** (Organomercurial lyase)
  - 651 n.t., 217 a.a
  - \textit{Staphylococcus aureus}

- **Hpt** (Hygromycin phosphotransferase)
  - 1,468 n.t, 332 a.a
  - \textit{Streptomyces hygroscopicus}
Confirmation of Inserted Genes and traits

Gene Expression and Selection of merB1 and merB4 line

<table>
<thead>
<tr>
<th>Control</th>
<th>Transgenic</th>
<th>Hyg40 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>B1</td>
<td>B2</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>B4</td>
</tr>
<tr>
<td></td>
<td>B5</td>
<td></td>
</tr>
</tbody>
</table>

PCR amplification

Northern blot analysis

Resistance of mercury

[Methylmercury chloride (MMC) $\text{CH}_3\text{HgCl}$ 1uM,
Phenylmercuric acetate (PMA) $\text{C}_6\text{H}_5\text{HgOCOCH}_3$ 5uM]
Inserted Gene Stability of GM *Solanum nigrum* L. in T3 generation

PCR based amplification of merB gene (650bp) from T3 GM plants using by specific primer set
(M, 1kb+ DNA ladder; -, wild type *Solanum nigrum* L.; +, GM *Solanum nigrum* L.)

Extraction of genomic RNA (B) and RT-PCR based amplification of merB gene (650bp) from T3 GM plants using by specific primer set
(M, 1kb+ DNA ladder; W, wild type *Solanum nigrum* L.; C, T-merB clone)
Comparison of biological traits and growth characteristics (flower, leaf, fruit, morphological traits / Seed germination, Pollen germination, weight, length, length of roots)

Comparison of physiology: Total protein, accumulation of mercury, glutathion conc. Etc
  - Assay of mercury tolerance and accumulation of GM *Solanum nigrum* L. in T3 generation

Biological Comparability

- Sample collection (sink, source)
- Hyg. (35ppm) Selection of transformants
- Transplantation (7~10 days after germination)
- Treatment of mercury (by concentration)
- Mercury tolerance assay (7 days after treatment)
- Sample collection (sink, source)
- Distillation and Ethylation
- Applied to EPA Method 1630

Sample with mercury

GC-CVAFS HPLC Analysis

National Institute of Environmental Research
BACKGROUNDs
Potential Risks of Virus Resistant Plant w/ CPMR

1. Construction of model Transgenic plant, *N. benthamiana* expressing ZGMMV coat protein gene

2. Construction of one point CP mutant vector for identification of transcapsidation or recombination

3. Potential effect of GM *N. benthamiana* expressing ZGMMV coat protein gene after infection of CP mutant virus

4. Genome stability of virus population during serial passages in Non-GM and GM plant
   - Zucchini Green Mottle Mosaic Virus (ZGMMV)
   - Cucumber Mosaic Virus (CMV)
   - ZGMMV + CMV
Virus Coat Protein Transgenic Papaya Provides Practical Control of *Papaya ringspot virus* in Hawaii

S. A. Ferreira and K. Y. Pitz, Plant and Environmental Protection Sciences, and R. Manshardt, Tropical Plant and Soil Sciences, University of Hawaii; F. Zee and M. Fitch, USDA-ARS Pacific Basin Research Center; and D. Gonsalves, Department of Plant Pathology, NYS Agricultural Research Station, Cornell University

### Broad-Spectrum Resistance to Different Geographic Strains of *Papaya ringspot virus* in Coat Protein Gene Transgenic Papaya

Huey-Jiunn Bau, Ying-Huey Cheng, Tsong-Ann Yu, Jiu-Sherang Yang, and Shyi-Dong Yeh
The potential risk concerned by virus resistant plants through coat protein gene-mediated protection (OECD consensus document, 1996)

- **Gene flow**: either from plant to virus
- **Synergy**
- **Transcapsidation**
- **Recombination**
Construction of plant transformation vector (pZGCPGA748) containing ZGMMV CP gene for *Nicotiana benthamiana* transformation.
Fig. 31. (A) Plant regeneration from transgenic callus tissue. (B) Wild type *N. benthamiana* leaf disc exhibits uniform susceptibility to Kanamycin. (C) Rooting of transgenic *N. benthamiana* shoots. (D) Seeds of transgenic T1 plants transferred to MS-kanamycin medium. (E) Seeds of wild type *N. benthamiana* transferred to MS medium. (F) Seeds of wild type *N. benthamiana* transferred to MS-kanamycin medium.
Detection of transgene and expressed proteins from transgenic T0 and T1 plants

Fig. 32. Detection of transgene and expressed proteins from transgenic T0 and T1 plants. (A) PCR analysis with a primer pair (ZGCP UP/ZGCP DN) flanking the transgene insertion site in the N. Benthamiana genome to confirm transgene. (B) Western blot analysis confirmation of stable expression of the transgene in T0 plant. (C) PCR analysis with a primer pair (35SC UP/ZGMMV CP DN) flanking the transgene insertion in the N. benthamiana.
ZGMMV challenged

Resistant

Susceptible

National Institute of Environmental Research
Construction of CP mutant vector for identification of transcapsidation

ATG(cp)→StuI

2GCPmutant1

```
GGCGTTCTGAGCTACCCGGGTATTATAACAGGCCCTTTACTCTACCAGCGGTATTCGTGC
```

```
2G
GGCGTTCTGAGCTACCCGGGTATTATAACAGATGCCTTTACTCTACCAGCGGTATTCGTGC
```

1kb ladder

9.5kb

1kb ladder

6kb

3.5kb

National Institute of Environmental Research
Potential effects of GM plant after 10\textsuperscript{th} (300 times) serial infection of the mutant virus

- No symptom of disease
Effects of infection at each serial passage of virus on GM/Non-GM tobacco

ZGMMV amplification

Visual Inspection

야생종 담배

민감성 GM담배

저항성 GM담배
Confirmation of CP and RP by PCR and cloning
LESC for Information Management

- LMO Environmental Safety Center
Thank You!