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

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Contents

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)**

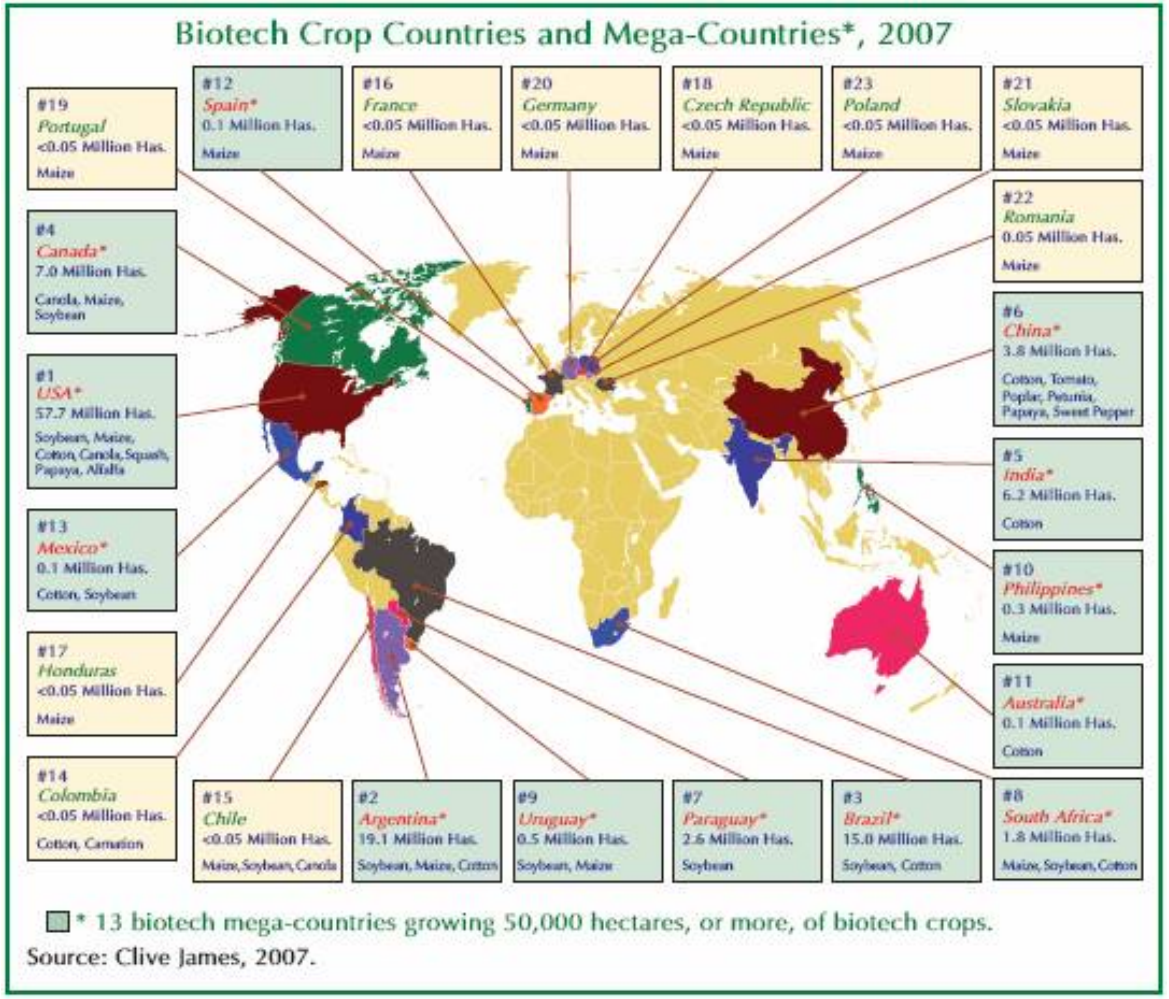
2000

- **Recombinant human antithrombin from GM goat, (ATryn, 2006)**
- Swine cloning for organ transplant (2002)
- Golden rice (2000)
- Blue rose (2004)
- **LM crops production (114.3 mha, 2007)**

향후

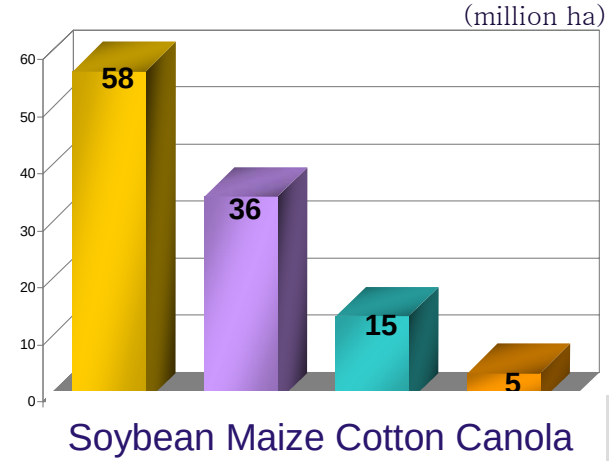
- Stress resistant Crops
- Bioenergy, Medical related R&D

GM CROPS Worldwide

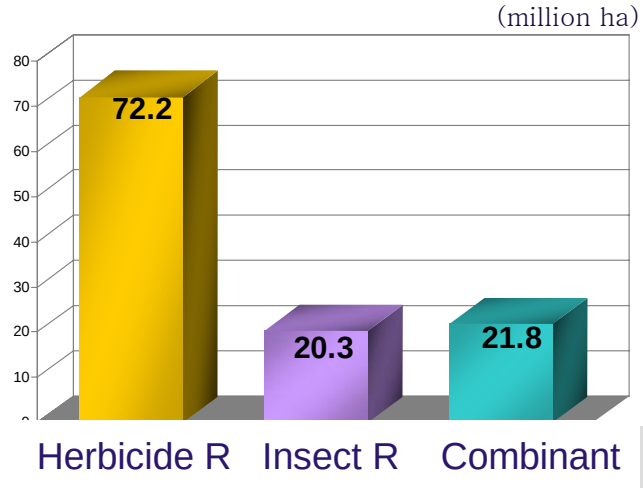


GM crops in 114.3 million ha, 23 countries in the world

- Crops [2007]



- Traits (2007)



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

- **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

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.

- **Monarch butterfly(1999-2001)**
  - larvae death from GM pollen (Bt maize)
- **GM canola in Japan(2004)**
  - GM canola growth beside the road from transportation

Technology and Market

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

- **StarLink corn(2000)**
  - corns approved for feed introduced to the ones for food network
- **LLRice601 (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	2000. 1 Adoptation of Cartagena Protocol on Biosafety to the Convention on Biological Diversity 2000. 9 Signed the Protocol
2001	**2001. 3 Adoption of LMO's Act**
2003	**2003. 9 International Effectuation of Biosafety Protocol**
2005-6	2005. 9, 2006.3 The Enforcement Ordinance of the Act
2007	2007. 12 The corporate notification of the Act
2008	**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 closured 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

Studies for Environmental Risk Assessment (ERA) of LMO

National Institute of Environmental Research

Model Studies of LMO for ERA

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

- **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**

ERA of Model Transgenic Weed for Potential Use of Bioremediation

BACKGROUNDS
Practicing the Whole ERA Procedure

1

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

2

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.

3

Effects on the Receiving Env.
-Effects on the Community: rhizosphere MOs, insects etc
-Gene transfer : target & non-target; relative sp. MOs, insects, etc

Construction of model Transgenic plant for RA

Function of Mercury Resistance Gene

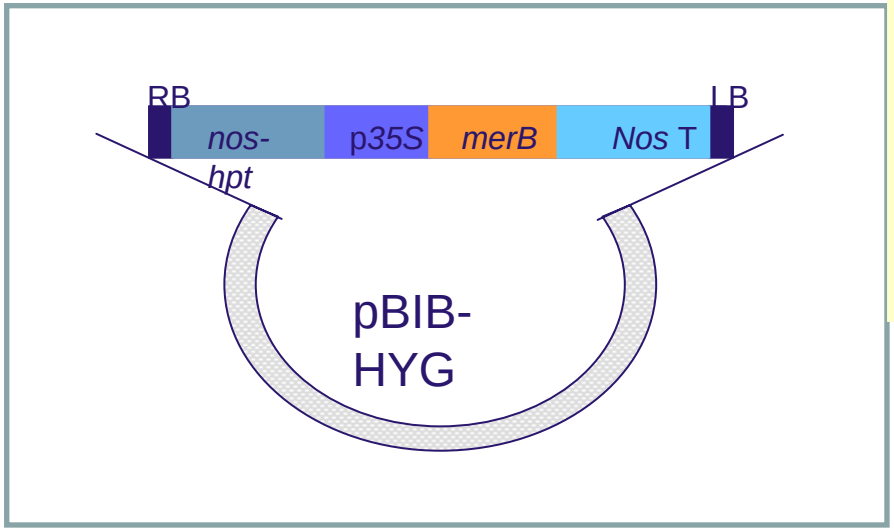
$MeHg^{+}$ (organic mercury) —***merB***→ Hg^{2+} (ionic mercury) —***merA***→ Hg^{0} (elemental mercury)

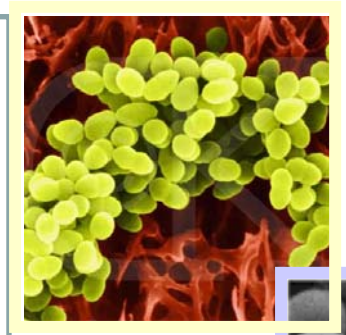
Parental Plant, Introduced Genes, Donor organisms & Vector

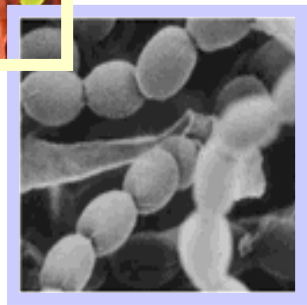
***Solanum nigrum* L.**
- Black nightshade, Common nightshade
- Seed breeding, annual weeds

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

Hpt (Hygromycin phosphotransferase)
-1,468 n.t, 332 a.a
-*Streptomyces hygroscopicus*

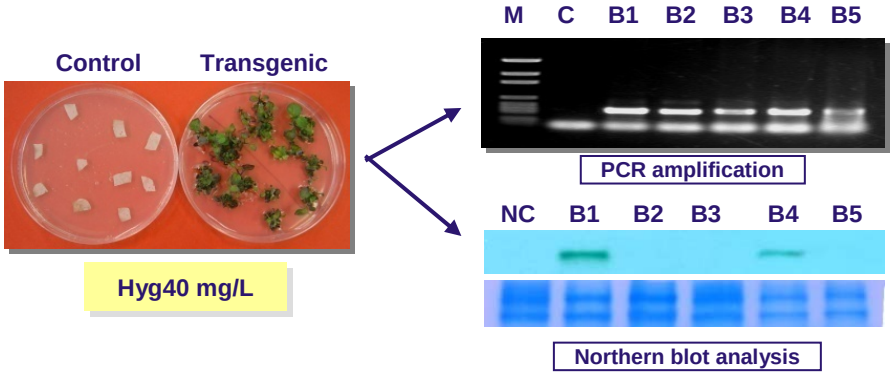


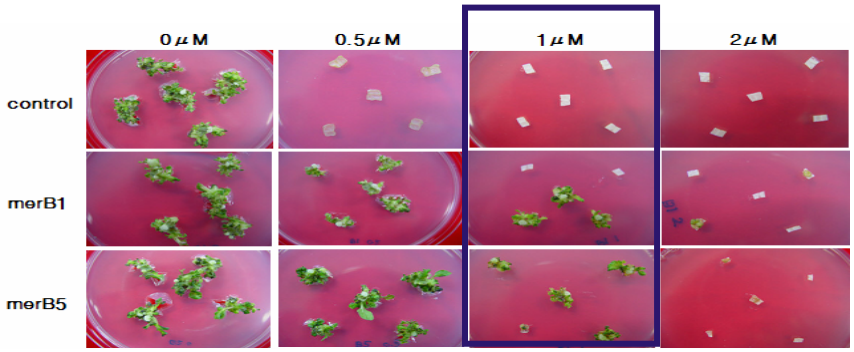




Confirmation of Inserted Genes and traits

Gene Expression and Selection of merB1 and merB4 line

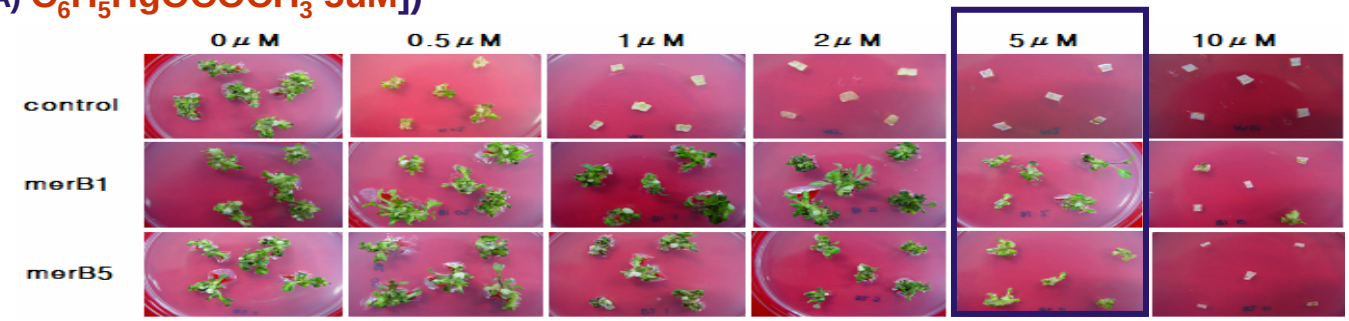




Resistance of mercury

[Methylmercury chloride(MMC) CH_3HgCl 1uM,

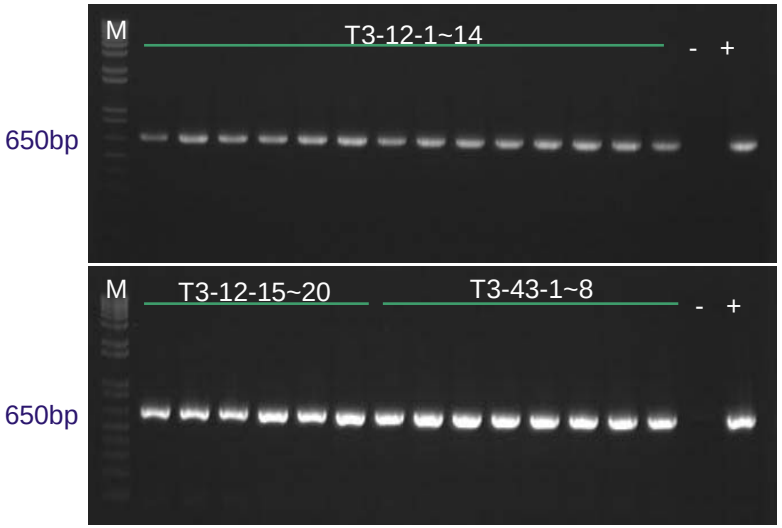
Phenylmercuric acetate(PMA) $C_6H_5HgOCOCH_3$ 5uM])

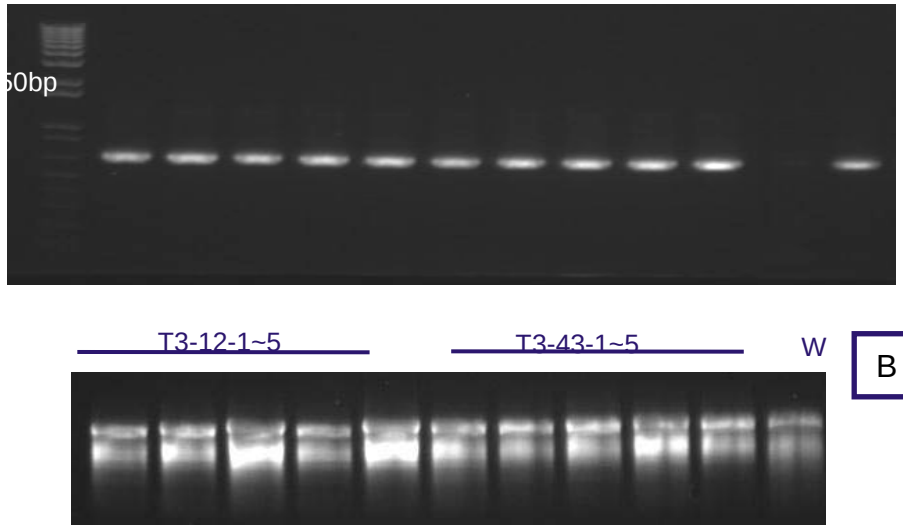


Gene Stability

Inserted Gene Stability of GM *Solanum nigrum* L. in T3 generation

A





B

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 nigum* L.)

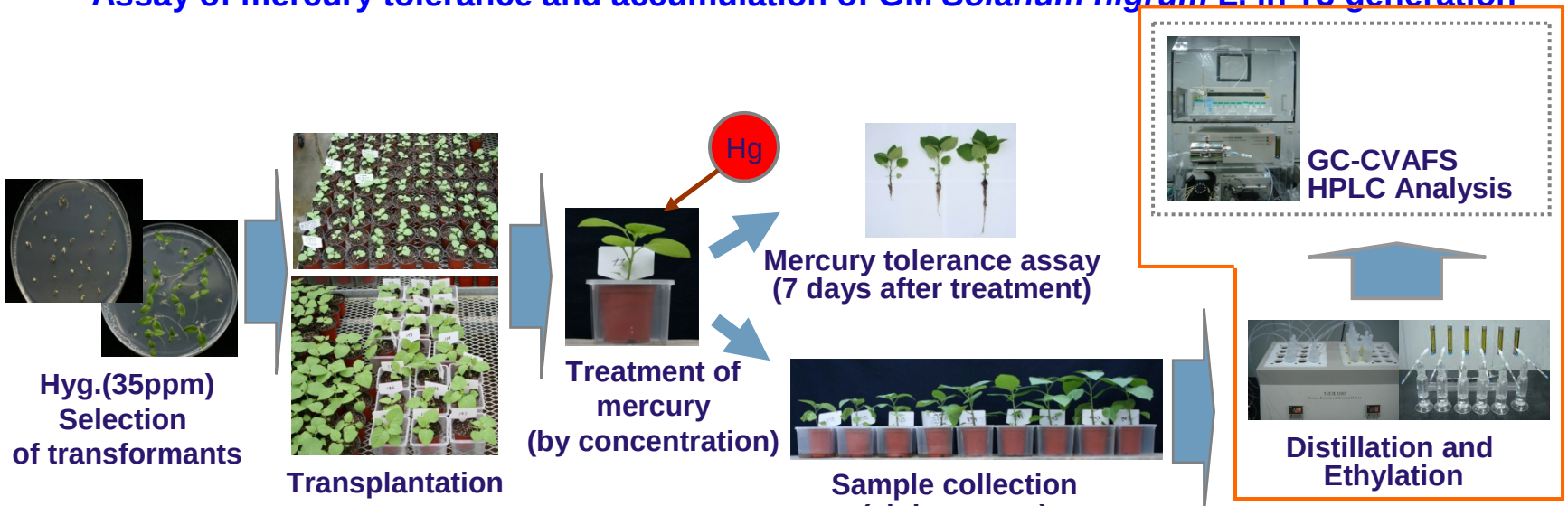
Extraction of genomic RNA (B) and RT-PCR based amplification of merB gene (650bp) from T3 GM plants using by specific primer set

(M, 1kb+ DNA ladder; W, wild type *Solanum nigrum* L.; C, T-merB clone)

Biological Comparability

- **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**



Applied to EPA Method 1630

ERA of virus resistant transgenic tobacco

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

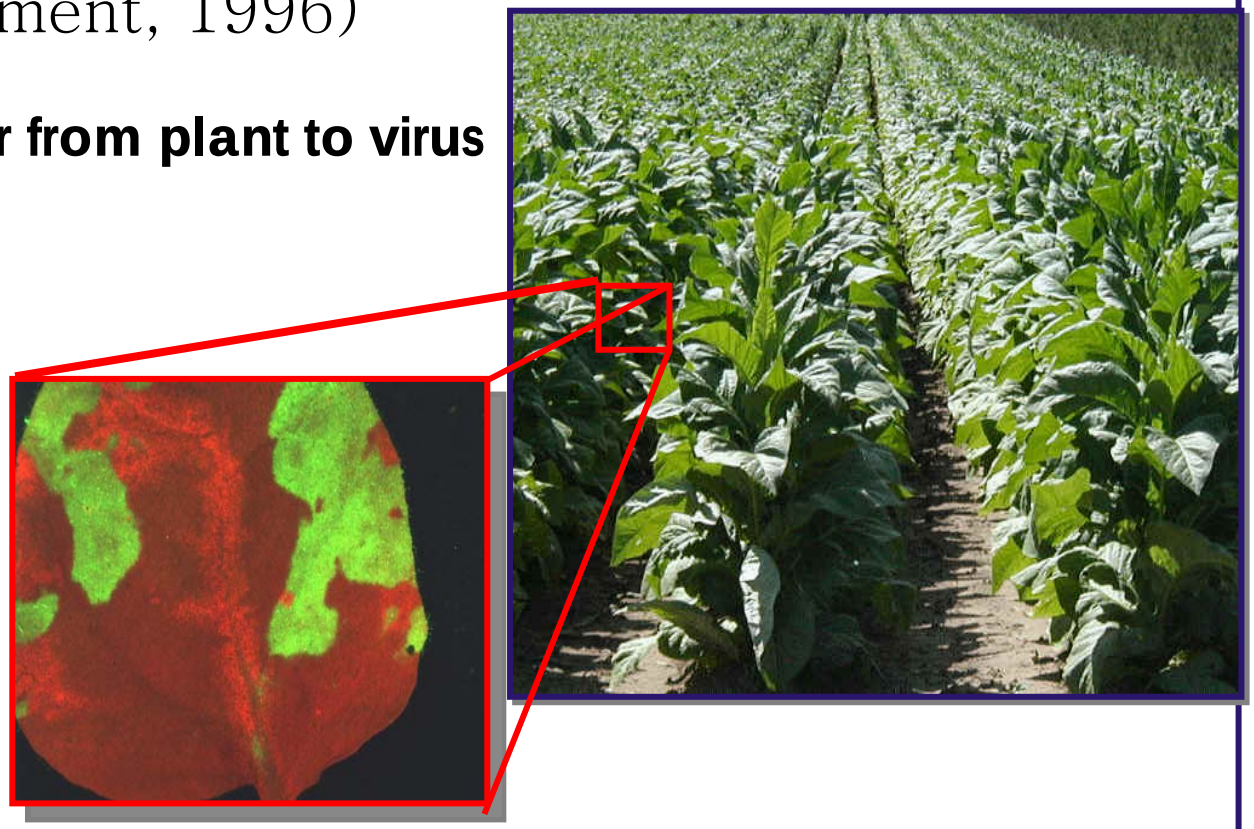
S. A. Ferreira and **K. Y. Pitz,** Plant and Environmental Protection Sciences, and **R. Manshardt,** Tropical Plant and Soil Sciences, University of Hawaii; **F. Zee** and **M. Fitch,** USDA-ARS Pacific Basin Research Center; and **D. Gonsalves,** Department of Plant Pathology, NYS Agricultural Research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

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

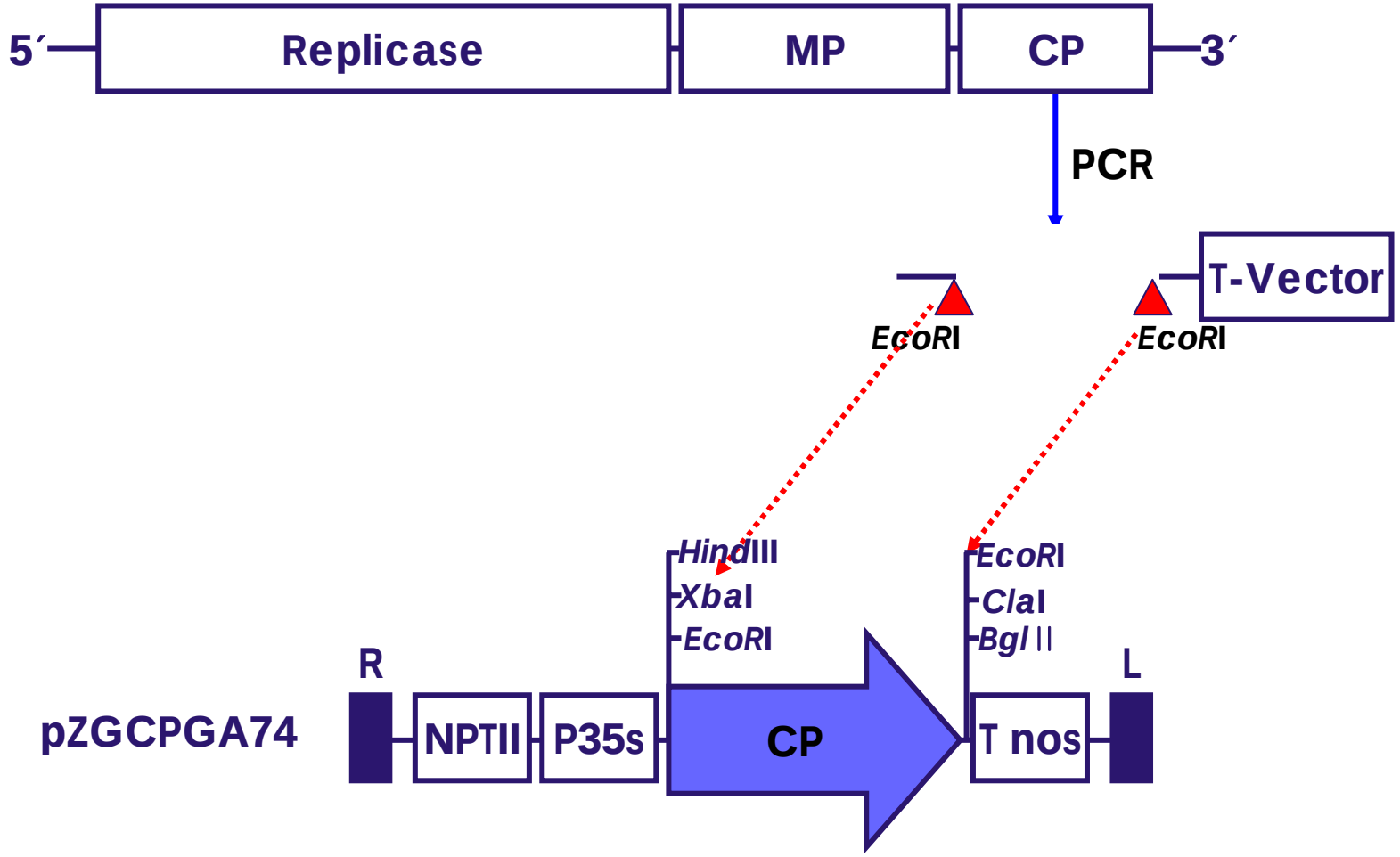
Huey-Jiunn Bau, Ying-Huey Cheng, Tsong-Ann Yu, Jiu-Sherng Yang, and Shyi-Dong Yeh

Potential risk assessment

- 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



Transgenic plants selection

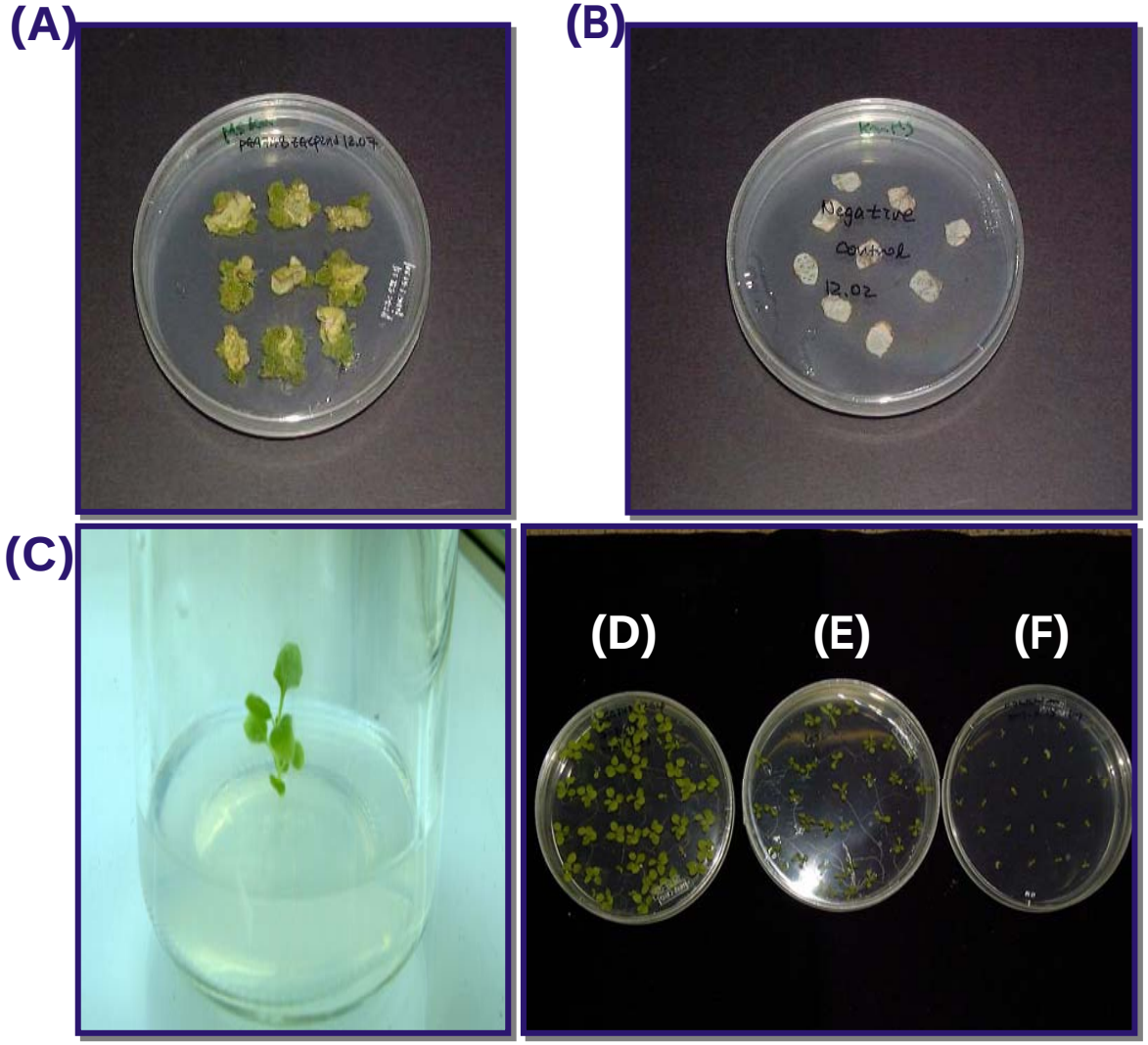
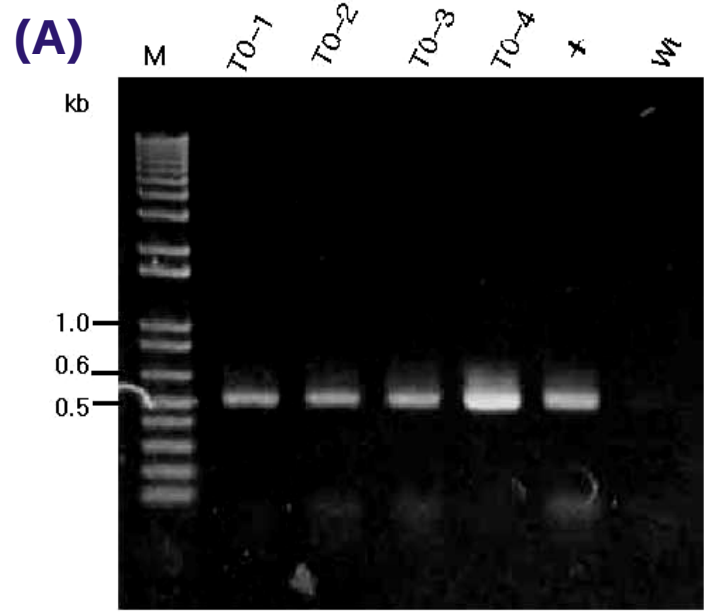
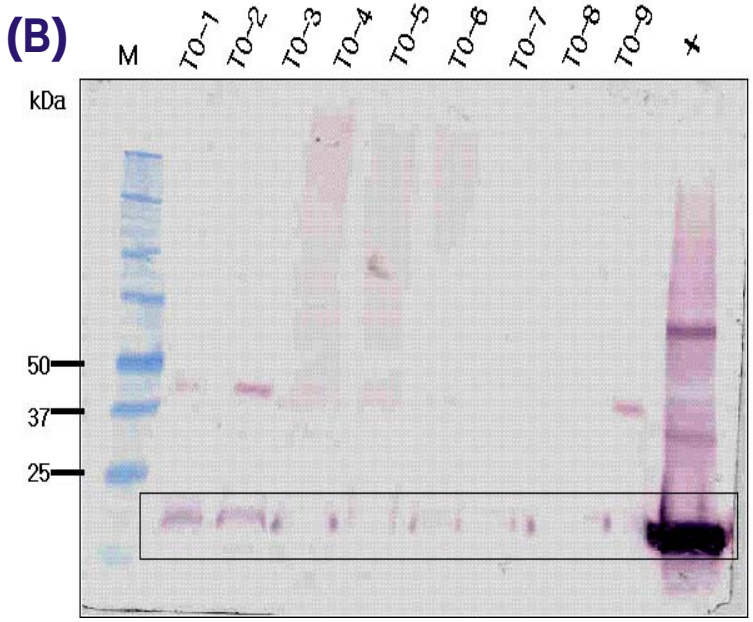


Fig. 31. (A) Plant regeneration from transgenic callus tissue. (B) Wild type *N. benthamiana* leaf disc exhibits uniform susceptibility to Kanamycin. (C) Rooting of transgenic *N. benthamiana* shoots. (D) Seeds of transgenic T1 plants transferred to MS-kanamycin medium. (E) Seeds of wild type *N. benthamiana* 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





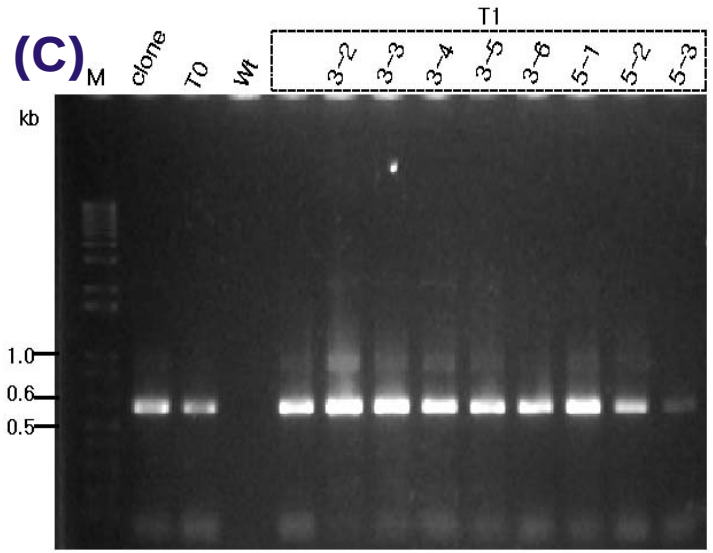


Fig. 32. Detection of transgene and expressed proteins from transgenic T0 and T1 plants. (A) PCR analysis with a primer pair (ZGCP UP/ZGCP DN) flanking the transgene insertion site in the *N. Benthamiana* genome to confirm transgene. (B) Western blot analysis confirmation of stable expression of the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

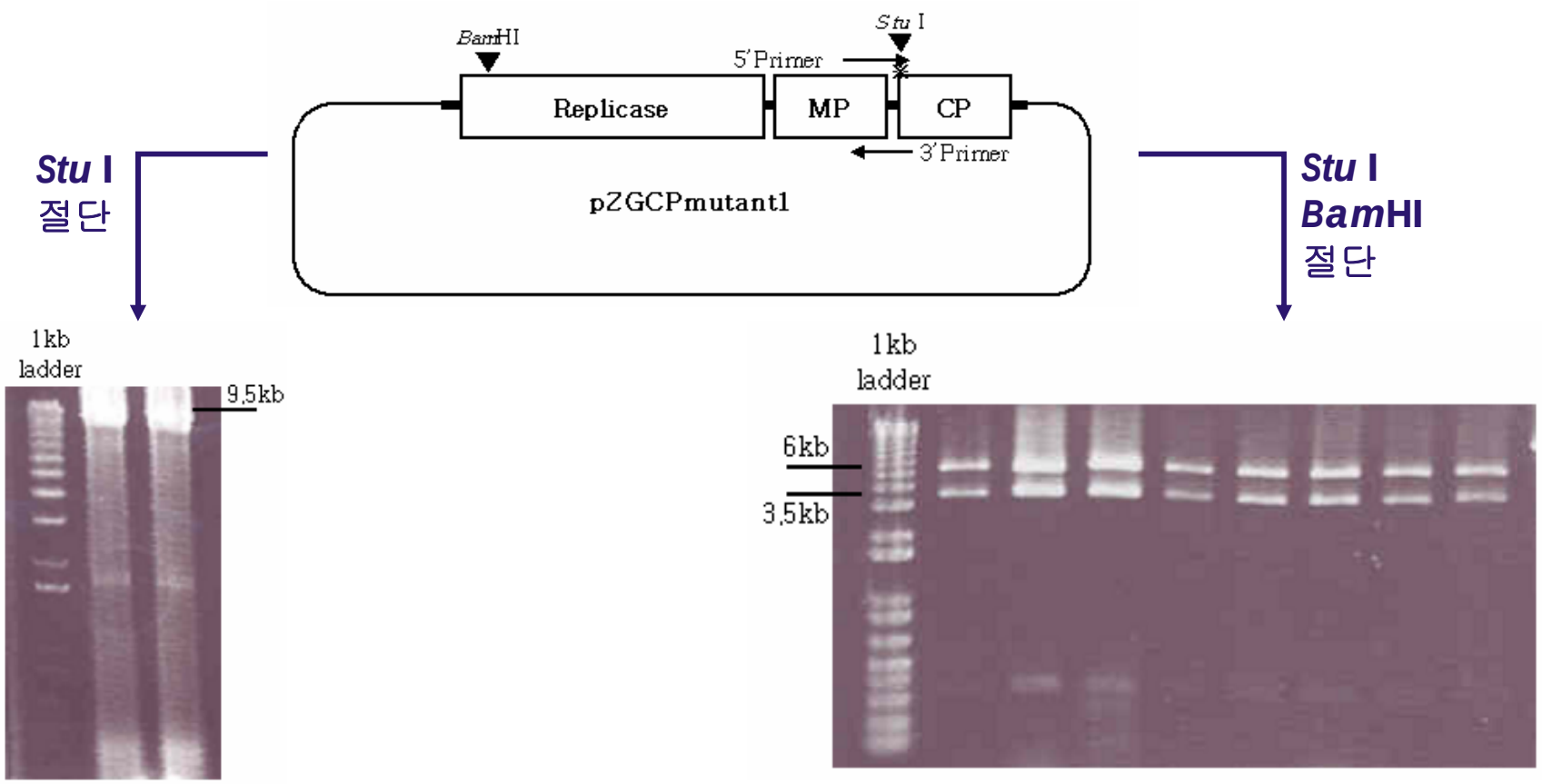
ZGMMV challenged

3_1

Resistant

Susceptible

Construction of CP mutant vector for identification of transcapsidation



ATG(cp)→StuI

```
2GCPmutant1 GCGCTTCTGAGCTACCCGGTTTATAACAAGGCCTCTTACTCTACCAGCGGTATTCGTTCG 518
2G          GCGCTTCTGAGCTACCCGGTTTATAACAAGATGCCTTACTCTACCAGCGGTATTCGTTCG 897
```

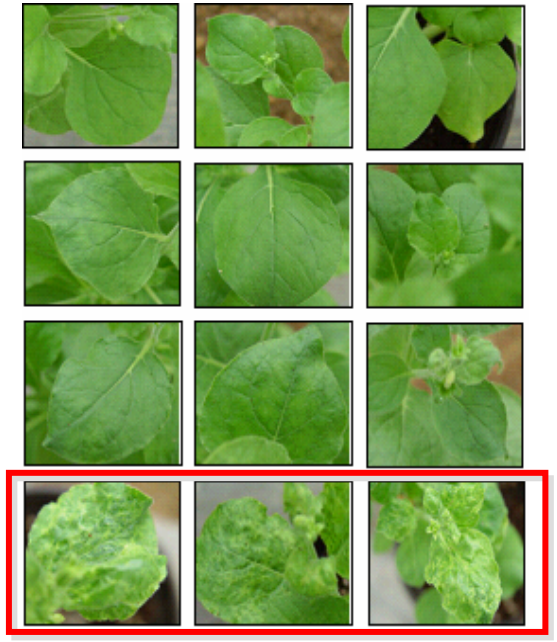
- Potential effects of GM plant after 10th (300 times) serial infection of the mutant virus
  - No symptom of disease

Symptom

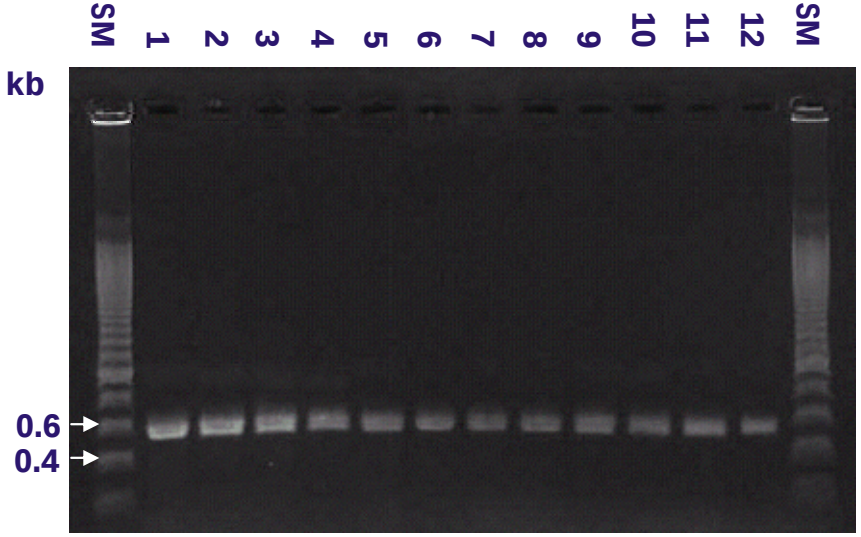
GM-R GM-S Wild

Mutant Inoculation

ZGMMV

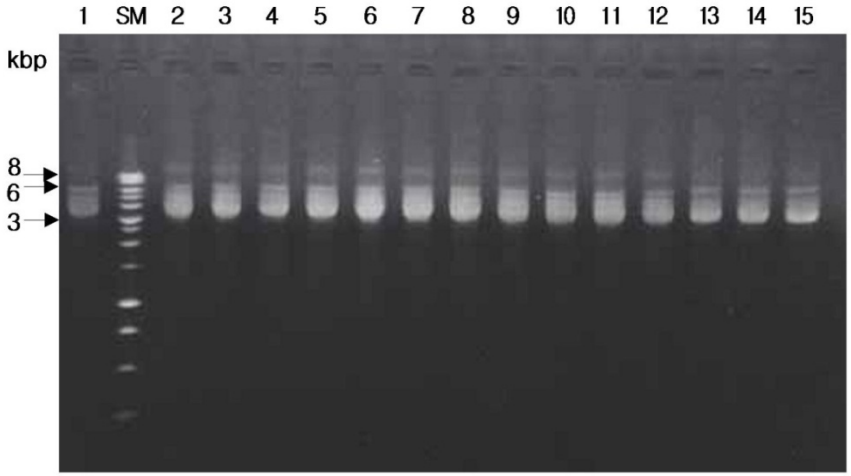


Evidence of Inoculation

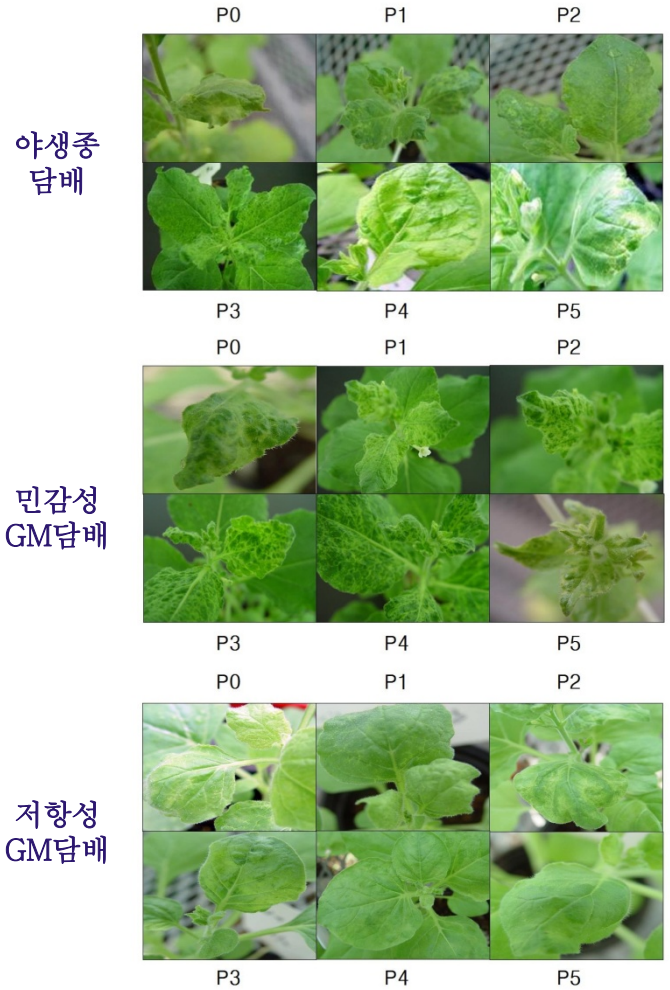


- Effects of infection at each serial passage of virus on GM/Non-GM tobacco

ZGMMV amplification



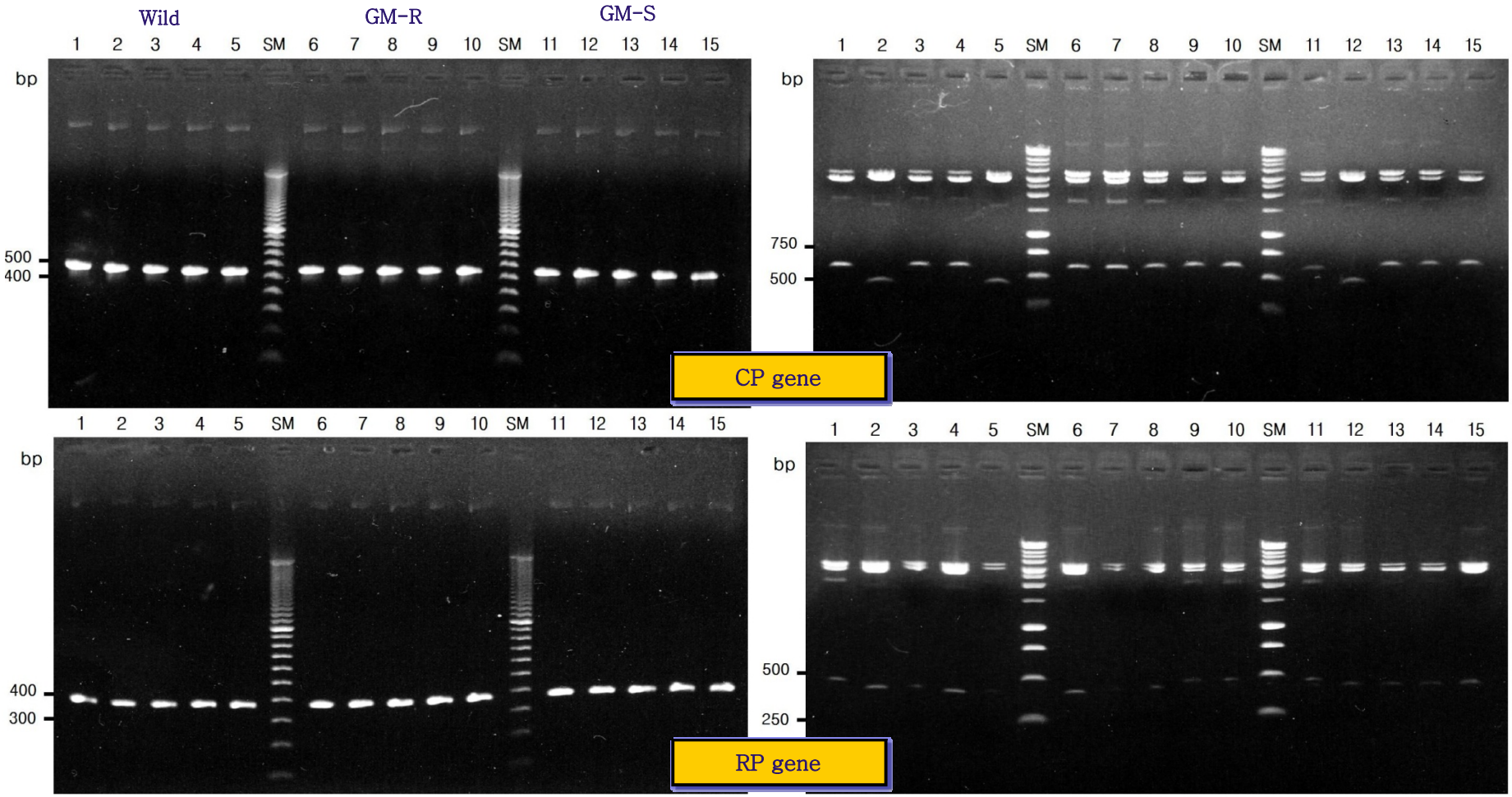
Visual Inspection



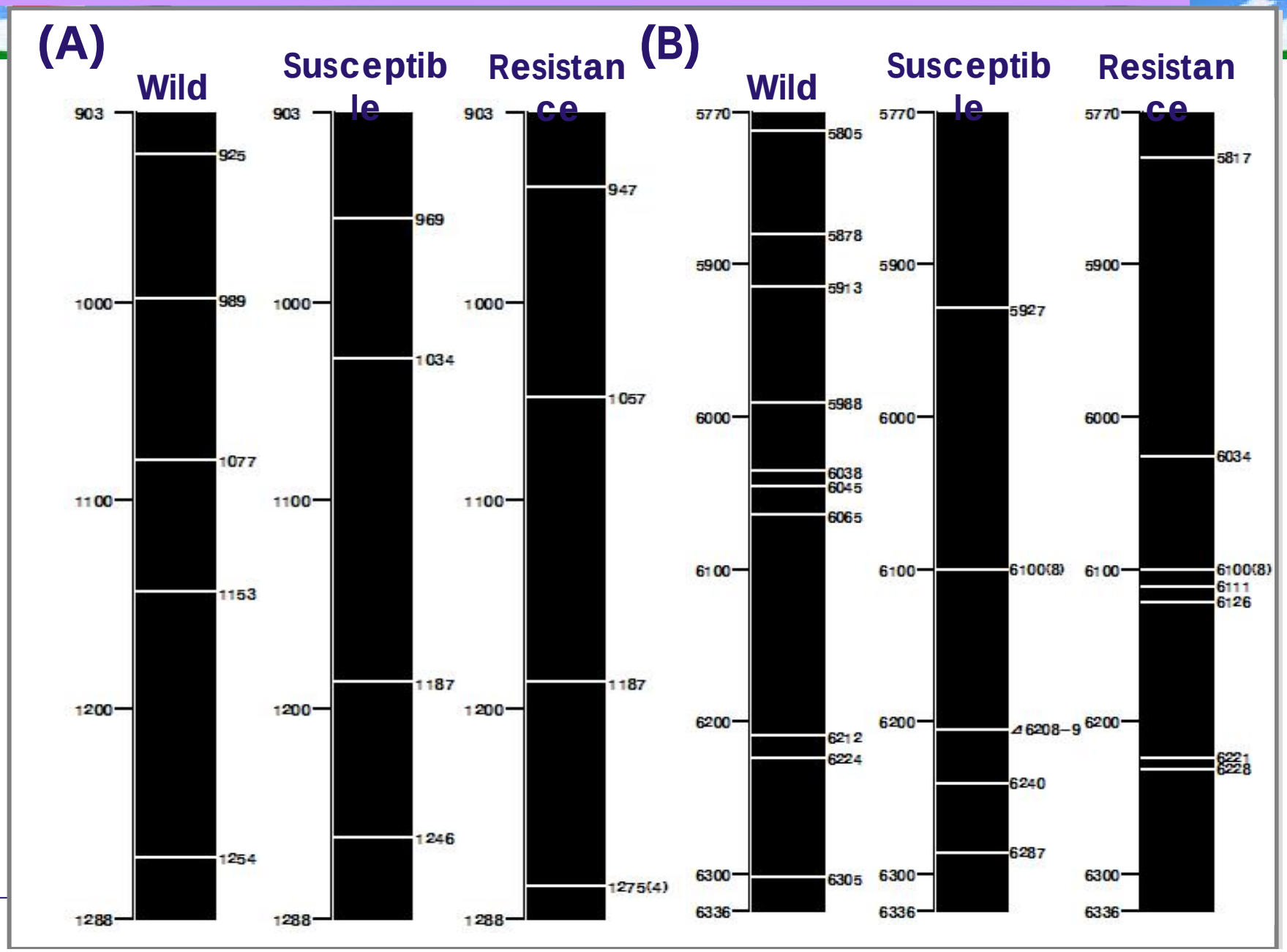
Confirmation of CP and R P by PCR and cloning

PCR

Cloning



ZGMMV 바이러스 + GM담배 / Non-GM담배 → ZGMMV 유전자변이율 조사



LESC for Information Management

- LMO Environmental Safety Center

Thank You !