Case Study In International Collaboration: Strategies for Management of the *Bean Golden Yellow Mosaic Disease*

J.S. Beaver, J.C. Rosas, S. Beebe, F. Morales, J. Faria, S. Singh, S. Temple, M.W. Blair, and D.P. Maxwell

Strengths of Collaboration

- Multiple Institutions
  - Universities, Federal Institutions, International Centers
- Multi-disciplinary approaches
- Continuity of effort and support by grants
- Free exchange of information and germplasm
- Use of most up-to-date methods

Topics

- Historical Background
  - Importance of WF-transmitted viruses in beans
- Early Research: 1970-1985
  - A. Costa, Brazil
  - J. Bird, University of Puerto Rico
  - Virus characterization – structure and N. A.
  - Breeding for resistance
- Approaches and achievements: 1985-present
  - Molecular characterization of viruses
  - Host-free period in Dominican Republic
  - Breeding for resistant cultivars; genetics of resistance
  - Marker-assisted selection
  - Transgenic approaches
  - Deployment of new cultivars
- Spin-off from BGYMV-research
- Conclusions (summary of impacts)

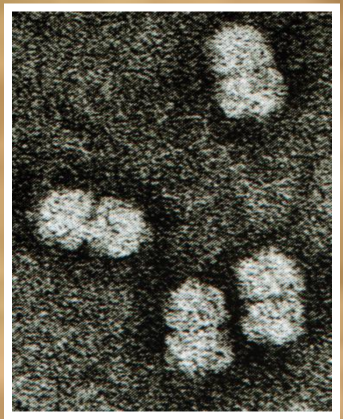
Whitefly-transmitted viruses

The bean golden mosaic virus complex
(Bean golden yellow mosaic virus)

Symptoms:
-- chlorosis (mosaic)
-- flower abortion
-- pod deformation
-- stunting

Bemisia tabaci

Disease



Importance

- BGM disease first observed in `60's by A.S. Costa in Brazil, and by the `70's was the major threat to bean production in Brazil. Also, by the `70s, it was present in Central America, Caribbean region and Mexico.
- The major problem in the Americas and Caribbean region!!

(F. Morales)

1980 in Argentina

F. Morales: "When I got to NW Argentina in 1980, 40,000 ha of Alubia beans had been plowed under" because of the infection by a WF-transmitted geminivirus (BDMV).

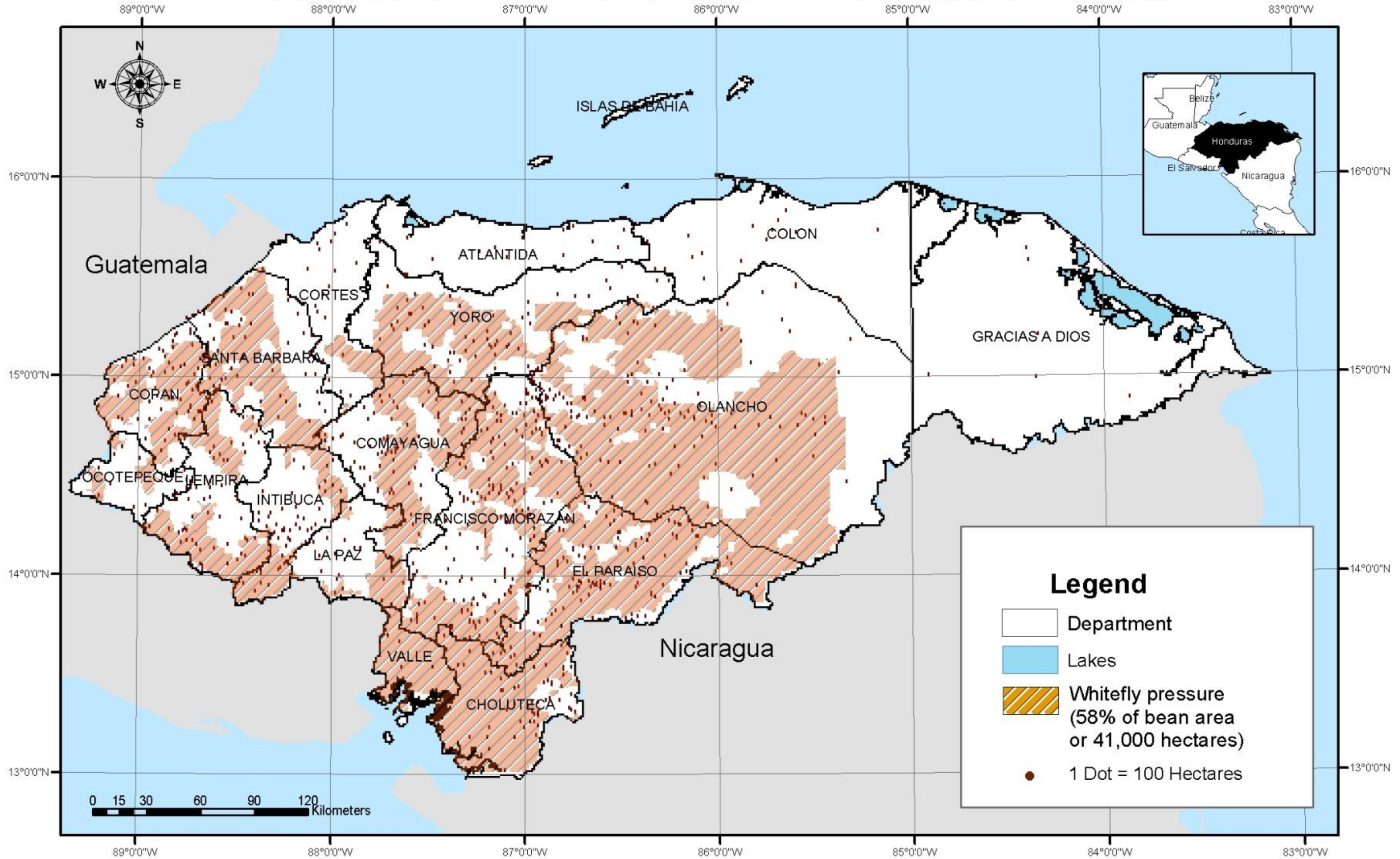
Estimated loss in 1980 dollars: $180 million

Importance – 1970's and early `80's

Dr. Francisco Morales (CIAT staff) wrote in 1994: "Bean golden mosaic virus is undoubtedly the main bean production problem in the lowland tropics (Latin America), particularly during the dry seasons."

Dr. Steve Temple wrote about his time in the `70's at CIAT: "I always considered BGMV the single most perplexing biotic challenge to increasing dry bean production."

HONDURAS: BEAN AREA BY WHITEFLY TRANSMITED VIRUS



Michigan State University
Bean Cowpea CRSP
Projection: UTM Zone 16
Geographic Coordinate System:
North American Datum 1927

Source:
IV National Agricultural Census 1993

Expert Opinion

Dominican Republic

San Juan Valley, losses of US $30,000,000 in `90-`92

Coyne et al., 2003

"devasting impact on resource-limited growers" (Coyne et al., 2003)

These growers could not afford insecticides to control the WF's.

Singh et al., 2000:

By early 1980's, "hundreds of thousands of hectares either were abandoned or could not be cultivated without the heavy use of systemic insecticides."

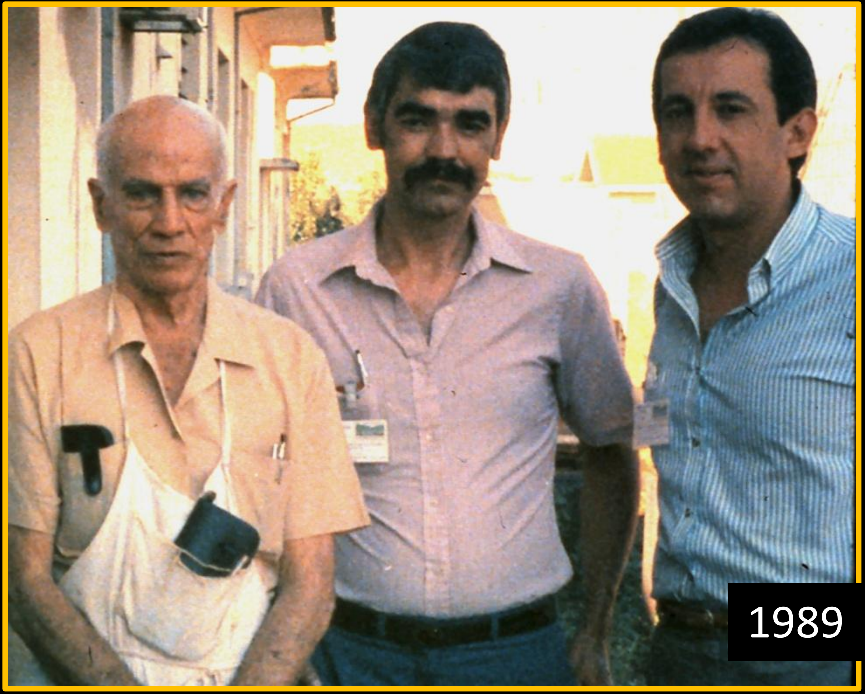
Factors contributing to increasing losses due to geminiviruses in the `80's

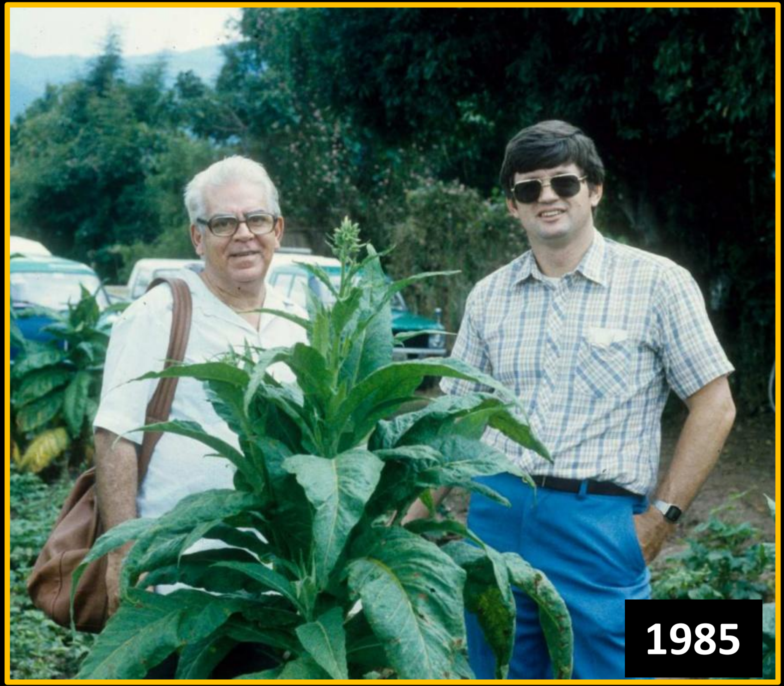
- ✓ Changes in agricultural practices
  - ✓ Continuous cropping of hosts (viruses and WF)
- ✓ Export crops
  - ✓ melons, tomatoes, soybeans
- ✓ New biotype of vector, *B. tabaci*, biotype B
  - ✓ reproduced on more plant species than native biotypes

Historical Developments

- A.S. Costa, Instituto Agronomico, Campinas, Brazil published first reports on WF-transmitted viruses infecting bean
- Julio Bird, University of Puerto Rico, studied host range of these viruses
- 1976, Galvez and Castano, at CIAT, published EM of virus particles – gemini-shape
- 1977, R.M. Goodman showed that genome was two circular ssDNA molecules, each ca. 2,600 nt

Early Researchers





A. Costa, J. Faria, F. Morales

Julio Bird, Charles Niblett

- Francisco Morales
- Shree Singh
- Steve Temple
- Steve Beebe
- K. Yoshii
- Maria Jose Zimmerman

Breeding for Resistance – 1970-86

- Brazil (70's): selection of resistant plants which later were found to be susceptible
  - Disappointing results (Pompeu and Krantz, 1977)
- CIAT (late 70's – 85): collaborative program with PROFRIJOL and ICTA (Cen. Am., Mexico, Caribbean) – trialing site in SW Guatemala
  - 7,000 accessions evaluated, no immune genotypes
  - three bl.-seeded types selected as parents (Porrillo Sinetico, ICA-Pijao, Turrialba-1) -- acceptable yield, with symptoms

(Yoshii, Galvez, Lyon, 1977)

CIAT-derived DOR (dorado) lines

Intercrossing of these parental lines resulted in release of three cultivars in Guatemala:

ICTA-Quetzal

ICTA-Jutiapan

ICTA-Tamazulapa

Losses: ICTA-Jutiapan (38%)

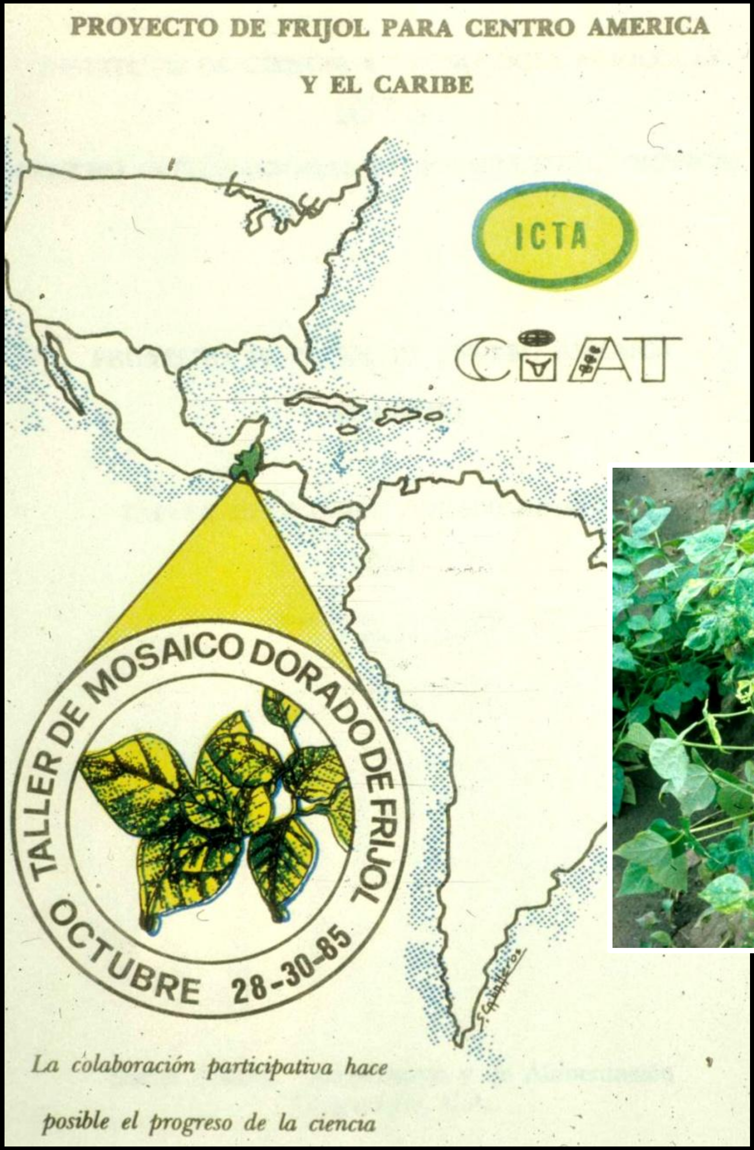
Rabia de Gato (86%)

Problems Still Existed

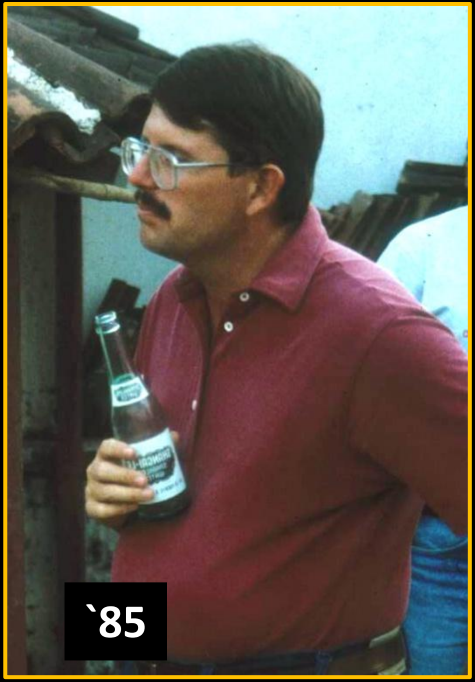
- Could still have high losses with DOR lines when WF populations were high
- No progress in red-seeded types (Costa Rica, Nicaragua, Honduras, Salvador)

CIAT- CRSP collaboration

- `81 – Bean Team Leader, Peter Graham, asked Steve Temple to represent CIAT at CRSP meetings
- >`81 Aart Schoonhoven, Bean Team Leader, CIAT, strongly supported CIAT/CRSP cooperation
  - `85 – CIAT organized BGMV meeting in Nov. in Guatemala


PROYECTO DE FRIJOL PARA CENTRO AMERICA
Y EL CARIBE
ICTA
CIAT
TALLER DE MOSAICO DORADO DE FRIJOL
OCTUBRE 28-30-85
La colaboración participativa hace
posible el progreso de la ciencia


`85


`85

Mid-1980's- New era of collaboration

- CIAT, EMBRAPA & Nat'l programs continued
- Bean/Cowpea CRSP **increased** efforts
  - University of Puerto Rico, Mayaguez – James Beaver
  - Escuela Agricola Panamericana, Zamorano, HN – Carlos Rosas
  - University of Nebraska - D. Coyne and J. Steadman
  - CESIAF, Dominican Republic – Freddy Saladin, E. Arnaud-Santana
  - University of Wisconsin-Madison - Douglas Maxwell
  - University of Costa Rica – Pilar Ramirez
  - University of West Indies – Wayne McLaughlin
  - USDA – Phil Miklas

New era of collaboration – expanded approaches

- Molecular characterization of the putative different WF-transmitted viruses (Begomoviruses) -- new detection methods
- Transgenic beans – anti-viral strategies
- New management strategies - host of the viruses
- Genetic studies and Marker-Assisted Selection
- Development and distribution of improved cultivars

Molecular Strategies - 1987

Sequence viruses

develop detection methods

Transgenic beans--

anti-viral approaches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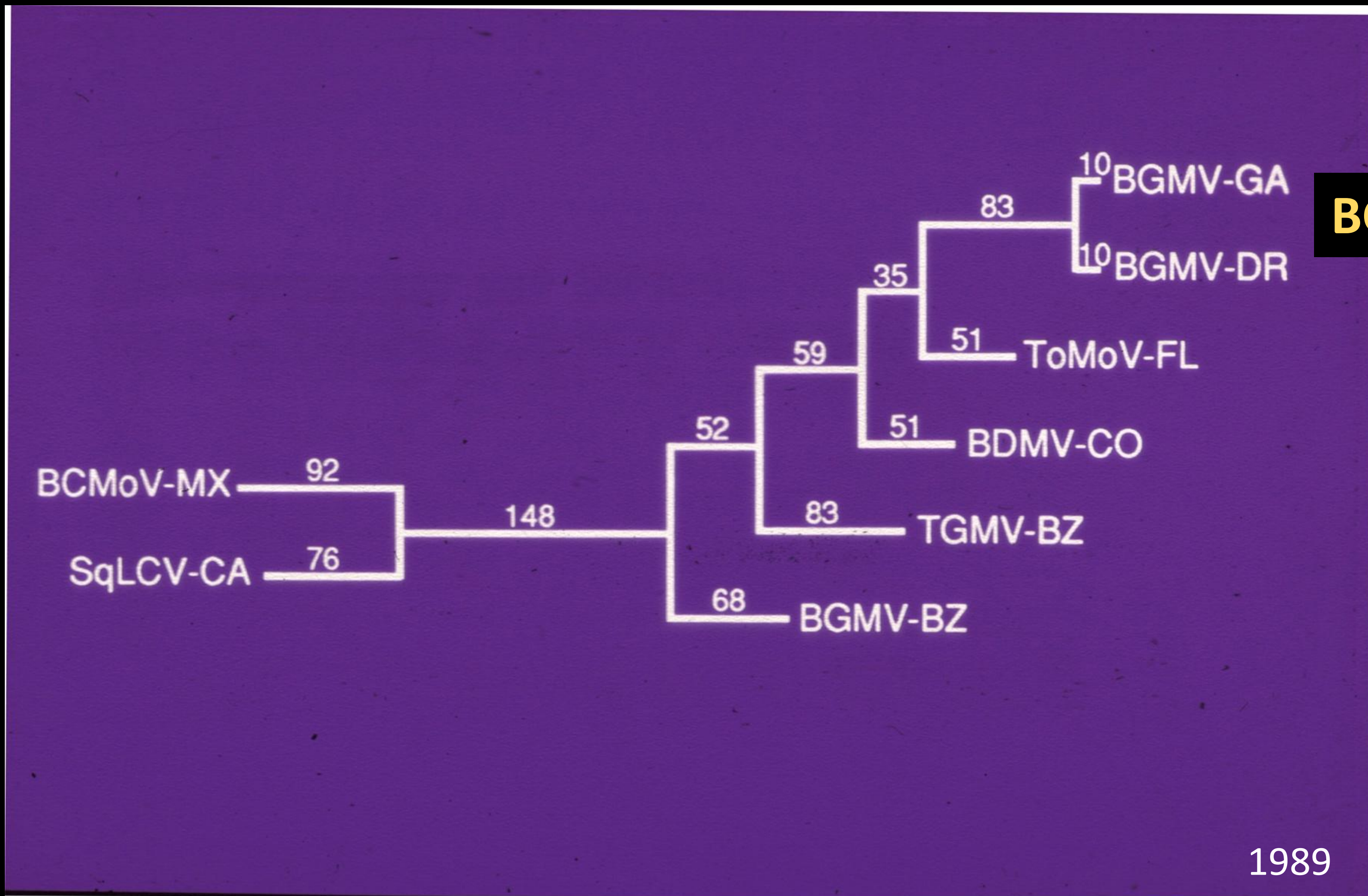
lat 1.292567° lon -74.032424° elev 3029 ft
©2009 Google
Eye alt 4248.03 mi

Cloning and Sequencing

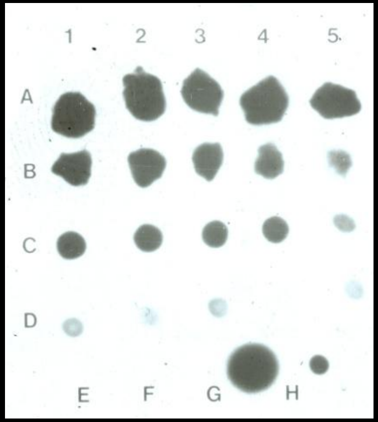


BGYMV

Detection Methods

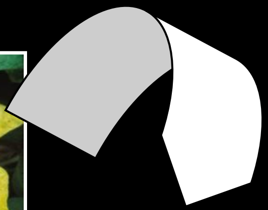
DNA hybridization methods

R.L. Gilberston, 1990, Dom. Rep.

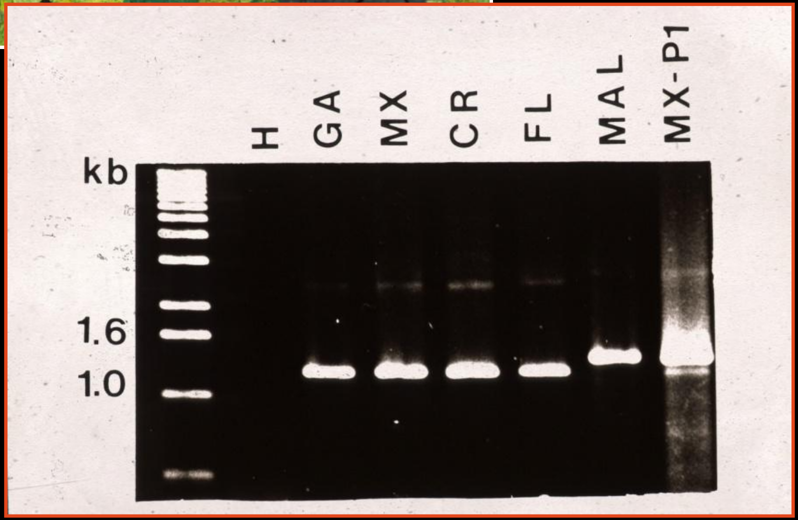


Gen. probe
Specific probe

1990 Costa Rica -- Maria Rojas



PCR
Primers
(universal)



Host Range Studies

Central America, Caribbean region, and Brazil

- Many weeds and crops checked
- Conclusion:

Beans most important source of inoculum!!!!

San Juan Valley, Dom. Rep.

- 60% of commercial bean production
- reduction in area by 7,000 ha
- Government passed **HOST-FREE** period law
  - 75 days no WF hosts
  - One bean growing season
  - Concentrated planting (5 Nov. to 15 Dec.)
  - High yielding, early maturing cv., PC-50

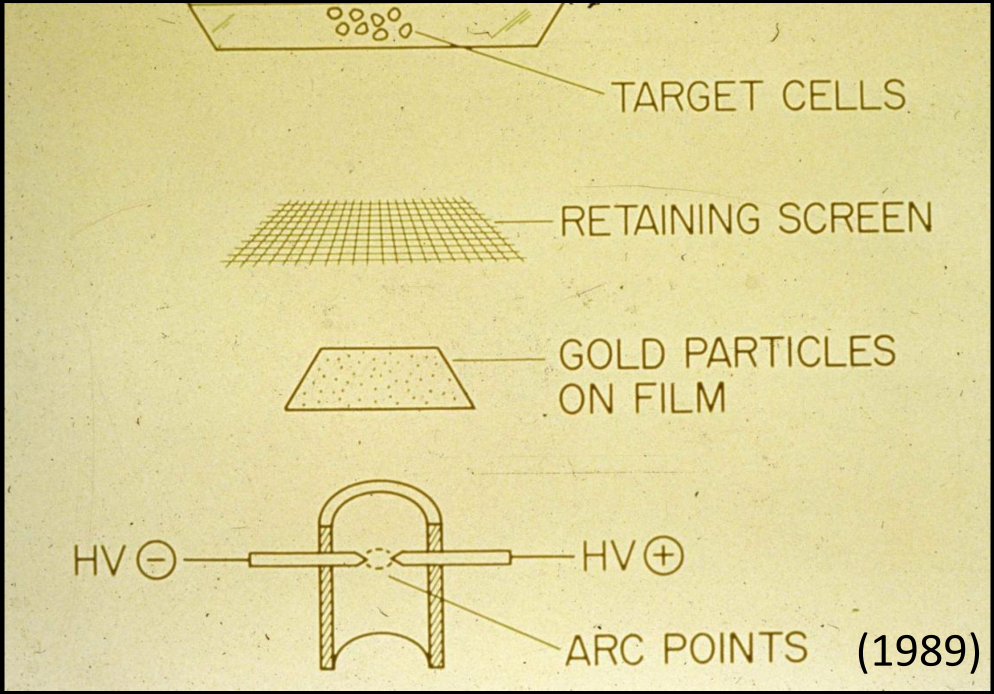
IMPACT: 90% production, sustainable

rDNA beans – resistant to BGMV

- Infectious DNA clones
- Anti-viral strategy
- Means for transforming beans with recombinant DNA

Infectious DNA Clones

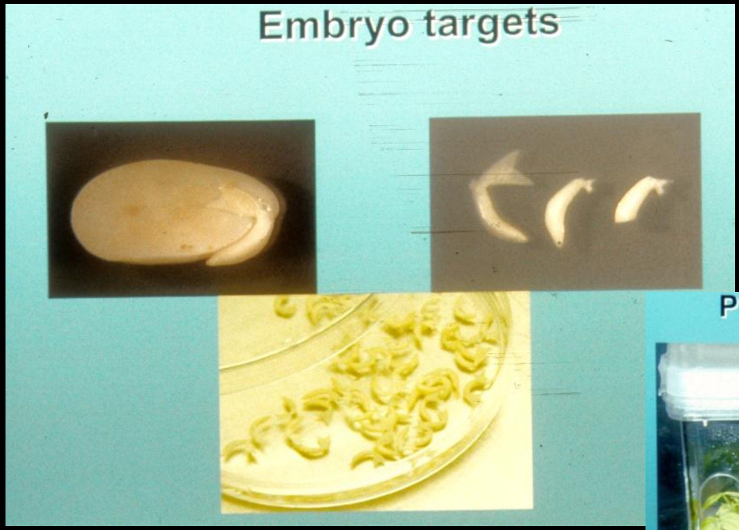
Agracetus, Inc. (D.R. Russell)



(1989)

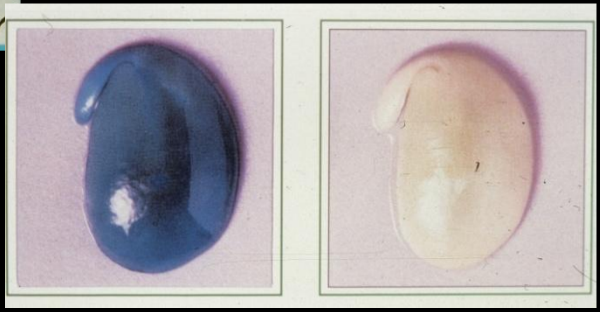
Biolistic gene insertion

Agracetus, Inc.



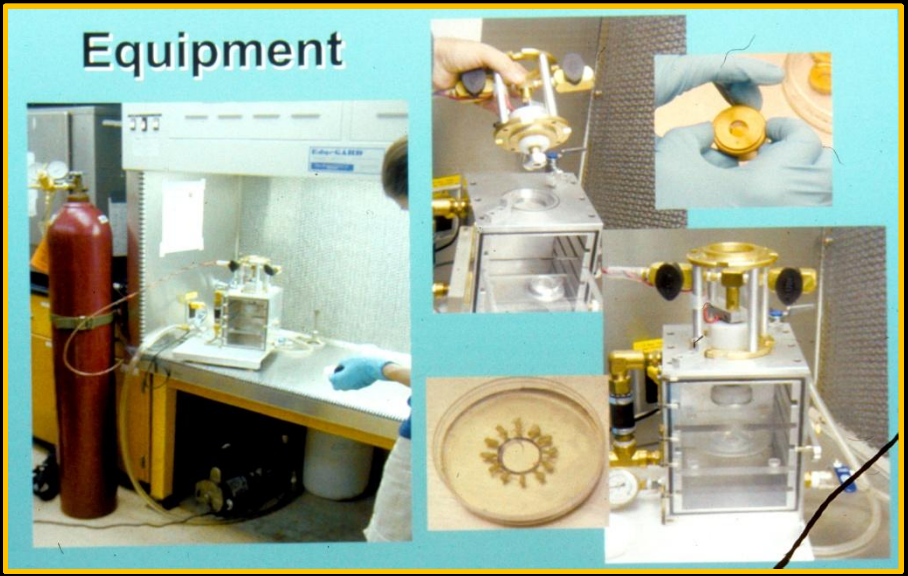
D. Russell's team, ca. '90

(Beta-galactosidase)



Anti-viral strategies

- Coat protein-mediated resistance
- Anti-sense to replication-associated protein
- Trans-dominant lethal against replication
- Post-transcriptional silencing (RNAi)



EMBRAPA-CENERGEN

Josias Faria
EMBRAPA-UW-CRSP

Anti-sense rep gene

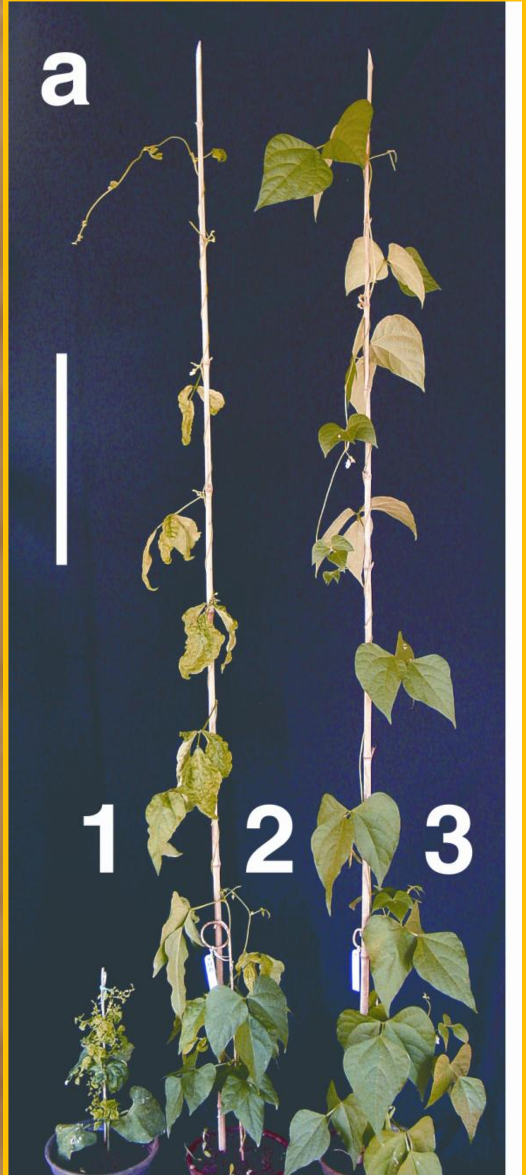
Trans-dominant lethal

Resistance to Bean golden mosaic virus in genetically engineered common bean mediated by RNAi

J. C. FARIA; K. BONFIM; E. O. P. L. NOGUEIRA; E. A. MENDES; F. J. L. ARAGÃO

CNAPF-CENERGEN

Support: Embrapa and FINEP (Financiadora de Estudos e Projetos – Ministry of Science and Technology)



NT T T

Greenhouse grown plants

Release of genetically engineered common bean – 2007 field;

RNAi plot remained free of infected plants

(T-beans were immune)

Transgene transferred to a commercial carioca variety by backcrossing

Major regulator issues!!!!

EXPECTED Com. Availability: 2014

Bean transformation, still difficult!!

Resistant Cultivars – `85 to present

(most important management tool)

- > `85 – CIAT; red-seeded types, DOR364
- Guatemala – new germplasm with R (CIAT)
  - A429
  - DOR303
- `88- Morales and Niessen (CIAT) – mechanical inoculation (Pl. Dis.)
  - variety of plant responses, e.g., dwarfing, chlorosis
  - transgressive segregation (F1 more R than parents)

Lucky find: in Guat. Beebe (CIAT) was screening large set of inbred lines, which had not been exposed to BGYMV - found A429 and DOR303

A429 (pinto type)

One of the most important sources of resistance

Pedigree

- Garrapato
  (Mexican Durango race)
  (reduced chlorosis, but had flower abortion)
- Porrillo Sintetico
  (meso-amer. Bl.-seeded)
  (tolerance, delayed symp.)

Lucky find: in Guat. Beebe (CIAT) was screening large set of inbred lines, which had not been exposed to BGYMV - found A429 and DOR303

DOR303 (red kidney type)
(dwarfing symptoms, reduced mosaic)

Pedigree

- Andean red kidney type
  (Red Kloud)
  (race, Nueva Granda)
  (chlorosis, but flowers and good pod load)
- Porrillo Sintetico
  (meso-amer. Bl.-seeded)
  (tolerance, delayed symp.)

Genetics of Resistance

- different resistant phenotypes, different genetics

CIAT scientists:

S. Beebe

S. Singh

F. Morales

Highest resistance from inter-gene pool crosses

Univ. of Puerto Rico, EAP, and USDA teams:

J. Beaver

J. C. Rosas

P. Miklas (USDA)

M. Basset (Univ. of Florida)

Students

M. Blair (now at CIAT)

C.A. Urrea

Greenhouse inoculations

Molecular Marker

Resistance cultivars, diff. seed colors

Genes conditioning resistance

Velez et al. 1998 (UPR, UFL – CRSP)

(reduced chlorosis)

- bgm-1 from A429 (parent: Garrapato)

  (partial resistance)

- bgm-2 from DOR303

Molina-Castaneda et al., 2004 - UPR-CRSP

resistance to pod deformation

(Bgp-1 in DOR482)

Molecular Markers

bgm-1 - MAS

RAPD marker (OR2) tightly linked to bgm-1 (A429, main source of resistance)

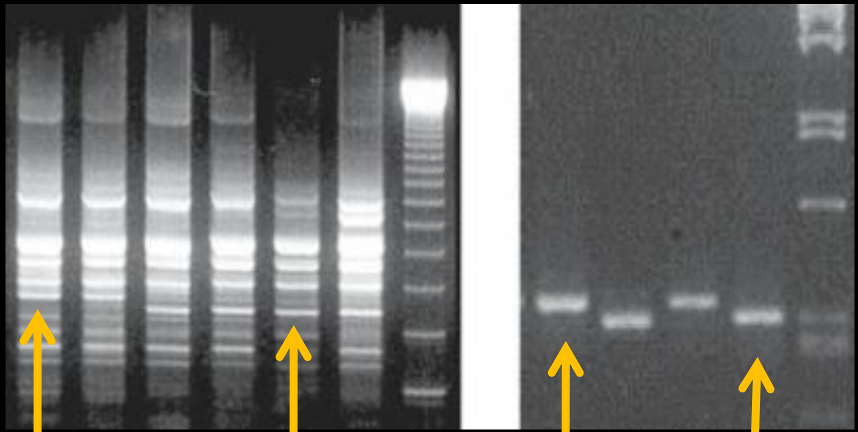
(Urrea et al., 1996 –UPR-USDA-CRSP)

SCAR marker (SR2)

Blair et al. 2007- CIAT

(located near end of chro. 5)

RAPD (OR2) **SCAR (SR2)**



S **R** **S** **R**

CIAT screens > 7,000 plants (bgm-1)

QTL SCAR marker for RAPD $SW12_{700}$

Miklas et al., 1996. Crop Sci

(Image of SW12 – gel)

Associated with delayed symptoms in DOR364 (Dorado), small red

Application of MAS

- SCAR marker (SR2) used for bgm-1

- SCAR marker QTL $SW12_{700}$ used for a major QTL SW12

both markers used routinely at CIAT, UPR, EAP and over 12,000 plants scored annually

Score in early generations without bioassay and can detect the recessive genes

Cultivars must have more than BGMV-resistance

Yield, drought, other virus-resistances, bacterial and fungal disease resistances, etc.

Steve Beebe: "Using markers has permitted accelerating the merging of the virus work into other streams of work. **This has been one of the big advantages of MAS for bgm-1/W12.** These markers do not assure high resistance but they increase the proportion of resistant lines greatly."

Novel Source of Resistance Genes

***P. coccineus* , G35172** Osorno et al. 2007

- bgm-3 (reduced chlorosis)
- Bgp-2 (low pod deformation)

(allelism tests: different than bgm-1, bgm-2, and Bgp-1)

Resistances from *P. coccineus* in breeding lines

- Black seeded – PR0247-49
- Small red – PR9771-3-2
- White -- PR0157-4-1

Breeding lines (MAS)

Participating institutions

CRSP (UPR, EPA, UNL)
National Programs
    Haiti
    Salvador
    Dom. Republic
    Costa Rica
    Guatemala
Universities – Cen. Am.
    Costa Rica
USDA
CIAT

Seed types (registered)

- Black (2)
- Small red (2)
- Red mottle (3)
- Light red kidney (1)
- Pinto (1)
- White (1)

TOTAL = 10

CRSP: BGYMV-resistant cultivars

Participating institutions

CRSP (UPR, EPA, UNL)
National Programs
- Haiti
- Salvador
- Dom. Republic
- Costa Rica
- Guatemala

Universities – Cen. Am.
- Costa Rica

USDA
CIAT

Seed types (registered)

- Black (4) >14,000 ha
- Small red (7) >88,000 ha
- Pink (1)
- White (3) >600 ha
- Snap bean (1)
- Pole bean (1)

TOTAL = 17

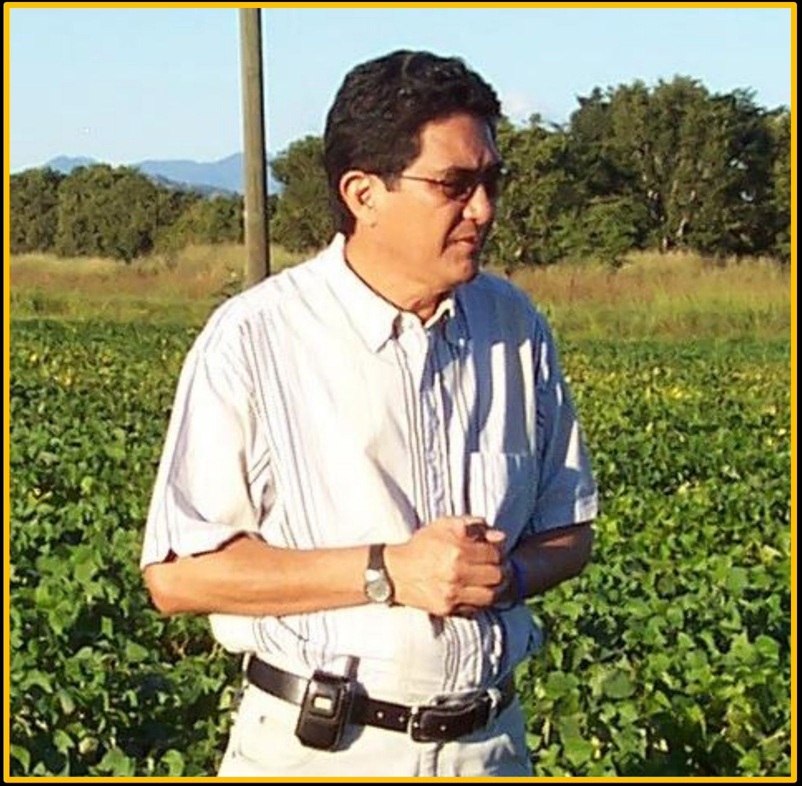
> 102,000 ha

Countries where CRSP R-BGMV cultivars are grown

- Caribbean
  - Puerto Rico
  - Dominican Republic
  - Haiti
- Central America
  - Costa Rica
  - Nicaragua
  - Salvador
  - Honduras
  - Guatemala

Foundation Seed Production and Distribution

EAP- Juan Carlos Rosas



Central America

- Governments (Nat'l Res. Inst.) - 90% EAP-CRSP cv.'s
  - Honduras, El Salvador, Nicaragua
  - Guatemala (2010)
- Private institutions
  - Zamorano
- Private companies
- Farmer organizations
- Individual farmers
- NGO's

Government programs

Mostly (90%) EAP-CRSP cultivars

- 25-50 lbs/farmer
- Some fertilizer

- **>120,000 farmers**

Haiti

CRSP Cultivars

- International organizations
  - FAO (DPC-40 , bl. Seeded, (BGYMV-BCMNV, purchased all seed, 30 March 2010)
  - USAID
  - CIDA
  - others
- **>20,000 farmers**

(Emmanuel Prophete)

Food Security – more dependable cultivars and high yield

- Estimated yield increases (75 kg/ha):
  - **7.5 million metric tons**
- Estimated meal-days (55 g/day):
  - **140 million meal-days**
- Dollar impact (150 lb/ha; $30/100 lb):
  - **$4.5 million**

Mechanism for Communication

- Central America
  - PCCMA

    Programa Cooperativo Centroamericano para el Mejoramiento de Cultivas y Animales

- North America
  - BIC (bean improvement cooperative) Meeting

    (www.css.msu.edu/bic/meetings.cfm)

Spin-offs

- Concept of HOST FREE period saved tomato industry in Dom. Rep. in 1995
- Begomovirus ID methods applied to other begomoviruses – many labs throughout the world
- Particle gun technology applied to many other begomoviruses – gene function studies
- Bean transformation – (still difficult)

Strengths of Collaboration

- ❑ Multiple Institutions
  - Universities, Federal Institutions, International Centers
- ❑ Multi-disciplinary approaches
- ❑ Continuity of effort and support by grants
- ❑ Free exchange of information and germplasm
- ❑ Use of most up-to-date methods

Aifi Wuriti
(Guat.)
black-seeded

Dedication of Presentation

In memory of
Dermot Coyne - UNL

In appreciation of
Pat Barnes-McConnell

Thank you

J.S. Beaver, J.C. Rosas, S. Beebe, F. Morales, J. Faria, S. Singh, S. Temple, M.W. Blair