

# La biotecnología y su impacto en la calidad de la semilla



Dra. Hilda Victoria Silva Rojas  
Postgrado en Recursos Genéticos y Productividad  
Producción de Semillas

8 de junio de 2009

## LÍNEA PRIORITARIA DE INVESTIGACIÓN

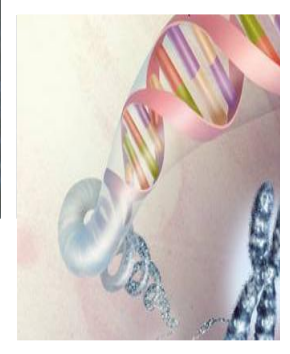


### BIOTECNOLOGÍA Microbiana, Vegetal y Animal (BiotecMiVA)



Es la línea de investigación que estudia y desarrolla tecnologías empleando organismos vivos, manipulados o no genéticamente, y sus productos para contribuir al desarrollo sustentable del sector agroalimentario y ambiental.

# LABORATORIO DE BIOTECNOLOGÍA DE SEMILLAS



## Las semillas son el punto básico de origen para la producción de alimentos



Alrededor del 90% de los cultivos alimenticios del mundo son sembrados directamente de semillas botánica

## La calidad de la Semilla

Física

Fisiológica

Genética

Fitosanitaria





**Calidad Genética**



**Identidad varietal**



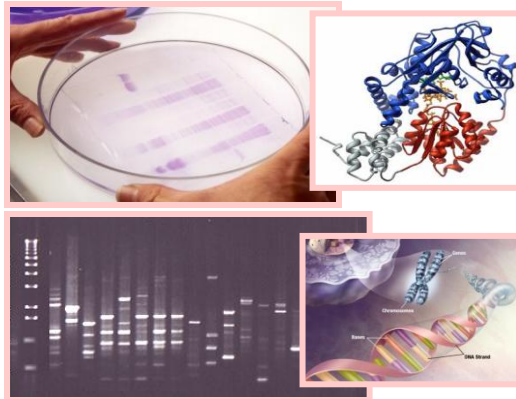
**Pureza genética**



**Marcadores morfológicos**



**Marcadores genéticos**

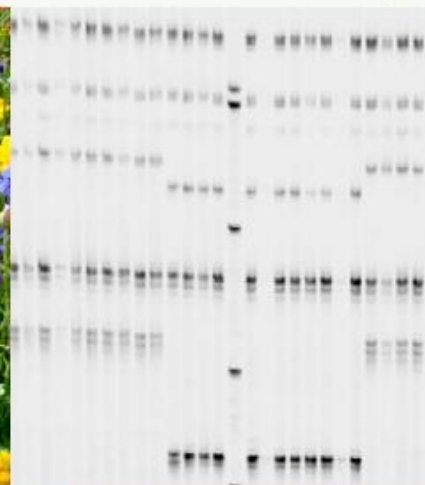


## Identidad varietal



# Seed Testing INTERNATIONAL

ISTA News Bulletin No. 135 April 2008



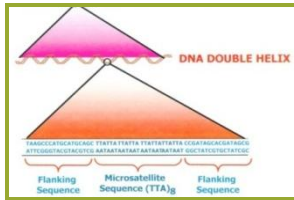
49 Quality control in wildflower seed production

26 DNA-based methods for variety testing

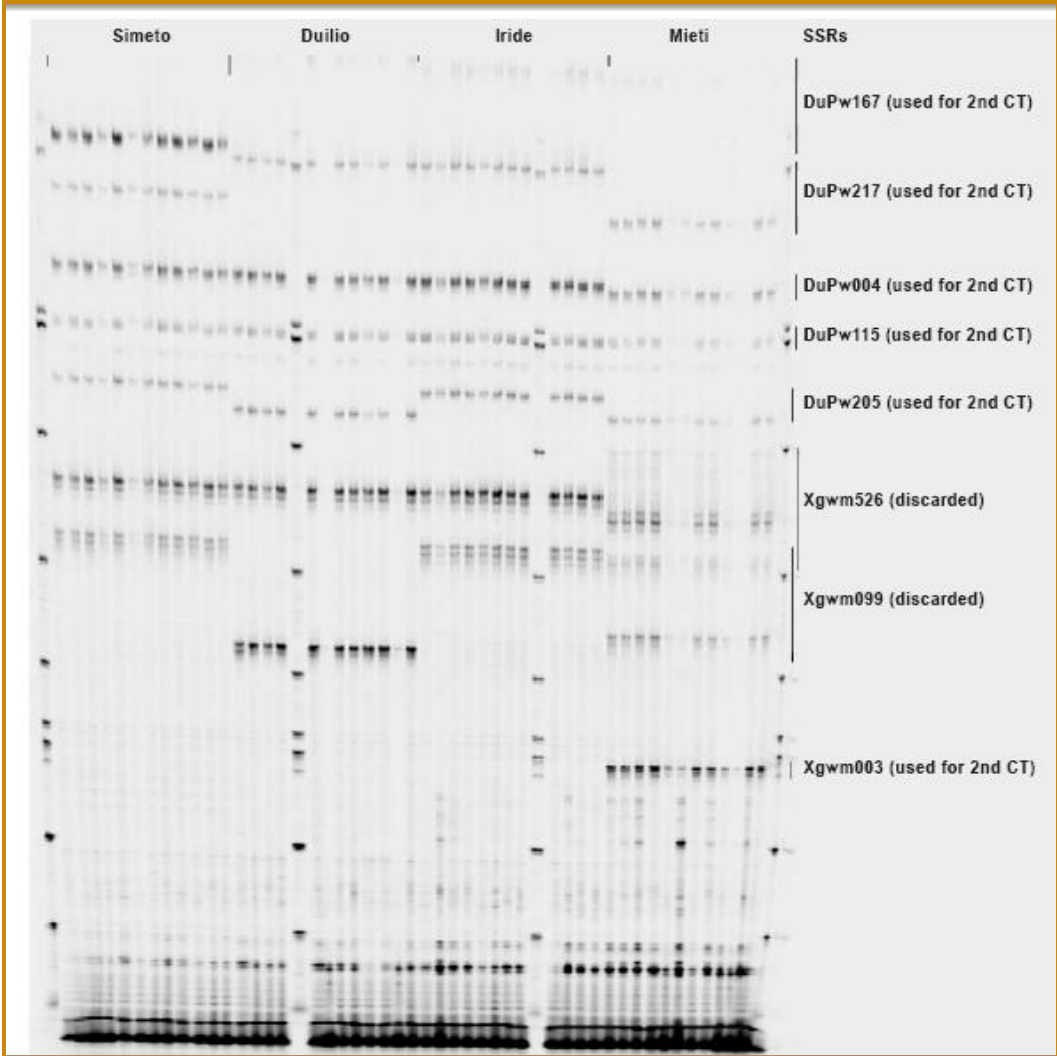
# Identidad varietal



Uso de microsatélites  
**Marcadores SSR**  
 Simple Sequence Repeat



```
TGAACAAACACACACACACACAC
ACACTGTGTGTTTGCCTTTATTCA
GTTTGTGTTGCCCTTGATCAGCTAA
TCCACATTACCTAGGACTTATTAGAA
ATGAAAGTCCCTGGACTCCACCCCA
ACACACTGAATCAGAACTCTGGCGG
CTGGGGGAGGGTGGGGGGCAGTAAT
TTAATTTGTTTTAAAAAGCTTCCA
GTGATTCTGGCGATGCACATTTATCTT
TAAAACTCCAATCCAATGATAAATG
TGTGTGTGTATGTGTGTGGTGT
GTGTGTTTGTCTCC
```







## RULES DEVELOPMENT

# First comparative test on DNA-based methods: final report of the Variety Committee Working Group

Dr. Emanuela Casarini<sup>1</sup> and Dr. Ana Laura Vicario<sup>2</sup>

<sup>1</sup>Member and <sup>2</sup>Vice-Chair of the ISTA Variety Committee

<sup>1</sup> Laboratorio di Ricerca e Analisi Sementi LaRAS,  
Bologna, Italy  
laras@agrsci.unibo.it

<sup>2</sup> Laboratorio de Marcadores Moleculares, Dirección  
de Calidad, Instituto Nacional de Semillas, Ciudad de  
Buenos Aires, Argentina  
alvicario@inase.gov.ar

The Variety Committee Working Group DNA-Based Methods was formed for the purpose of developing an agreed DNA-based approach for the checking of variety identity in species of interest, to be added to the ISTA Rules.

The aim of the first comparative test was to provide a list of DNA-based markers which could be used to distinguish varieties of *Zea mays* (maize), *Oryza sativa* (rice), *Triticum durum* and *Triticum aestivum* (wheat) and *Glycine max* (soybean). The aim was also to compare results between participant laboratories, and evaluate whether it is possible to obtain the same band patterns and allele sizes even when

obtain reproducible results among ISTA laboratories.

We consider simple sequence repeats (SSRs), also known as microsatellites, to be one of the best types of molecular markers for variety identification. SSRs are inexpensive, amenable to automation, co-dominant, independent of the environment, highly polymorphic, essentially unlimited and multiallelic, and provide coverage of the entire genome.

For each species, we aim to select a set of SSRs suitable for varieties from all parts of the world, and which, if necessary, may be complemented with additional subsets for specific regions.

In the future, a lab that wishes to perform a test using this list of SSRs will have the freedom to choose reagents, protocols and visualization methods to suit their instrumentation and other needs (tables 2, 3).

We believe that the checking of variety identity is the simplest and fastest way to

reaction (PCR) protocols, depending on the lab equipment available (table 3).

### Results

After the first comparative test, seven SSRs were selected for maize, six for wheat (fig. 1) and nine for soybean. No SSRs have yet been selected for rice.

Each crop subgroup decided on a set of appropriate SSRs, based on the following criteria:

- Markers easy to score: no or little stuttering, no faint bands and sharp alleles;
- Markers giving the same clustering among labs.

SSRs not fulfilling these criteria were discarded. The remaining SSRs will be used during the second comparative test. New SSRs will be proposed in order to obtain a set of about 12 SSRs per species.

### Conclusions and further steps

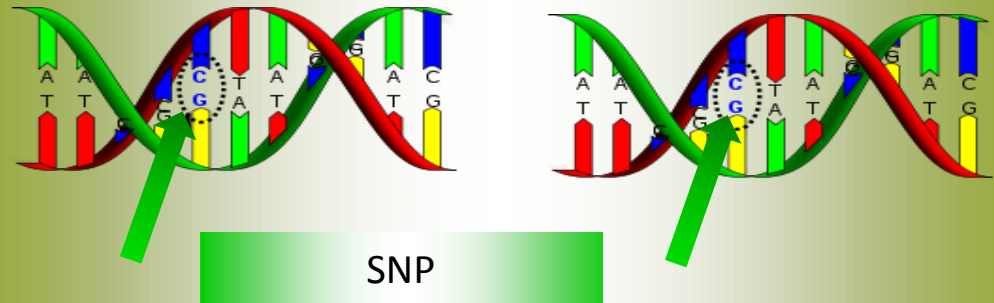
During this first comparative test, sets

## Pureza genética

Uso de  
**Marcadores SNP**  
Single Nucleotide Polymorphism



“.....We believe that the checking of variety identity is the simplest and fastest way to start, but we also see a need, in the future, for using this method for performing purity tests as well. For purity testing it may be necessary to validate other kinds of molecular markers, such as single nucleotide polymorphisms (SNPs)”.



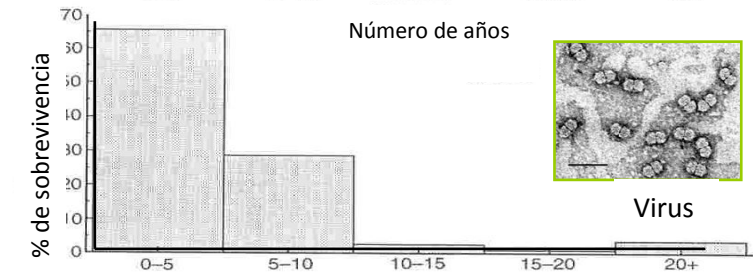
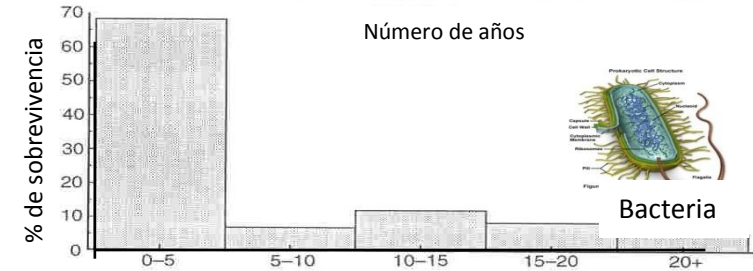
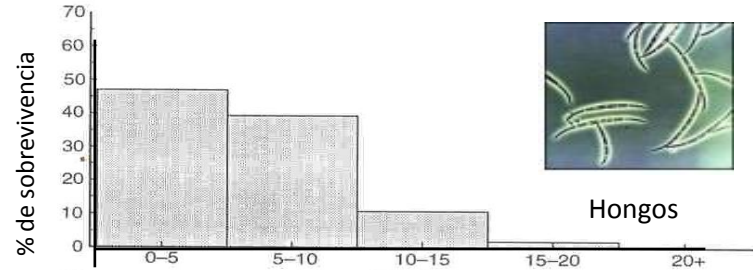
**Calidad fitosanitaria**



**Detección**

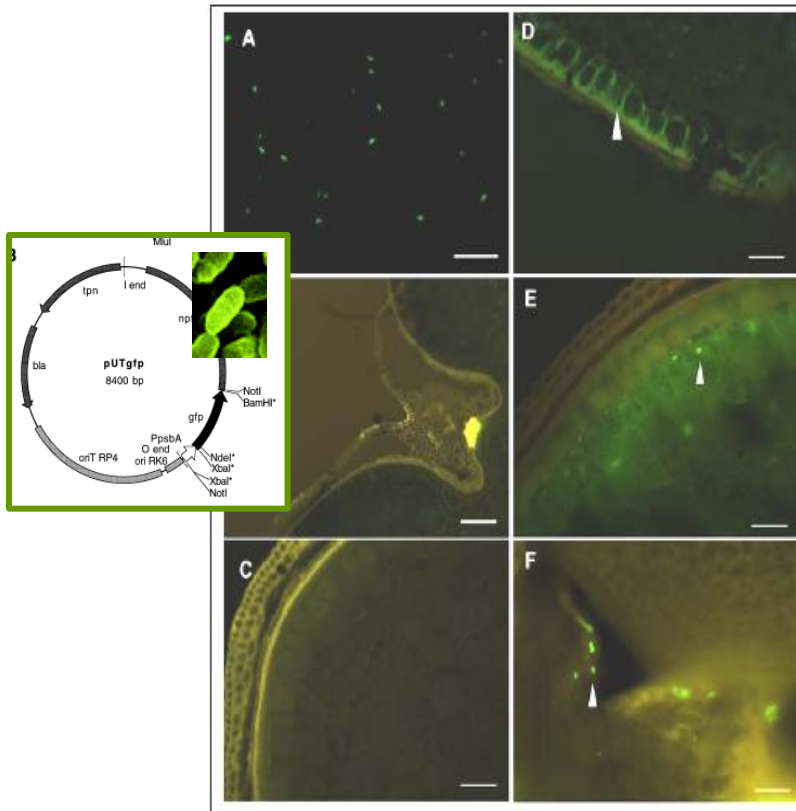


**Identificación**

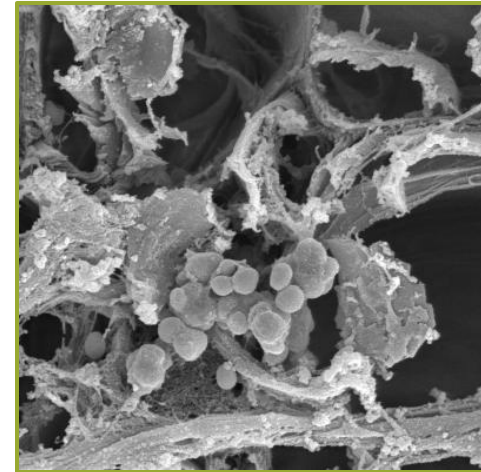


Número de años

➔ Localización y movimiento de *Pseudomonas syringae* dentro de la semilla de trigo



Proteína verde fluorescente

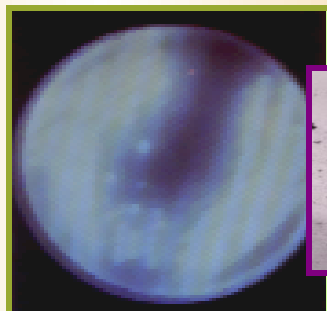


Reacción antígeno-anticuerpo

Microsc. Microanal. 13:298-299 (2007).

## Proyecto CONACYT I39319-B

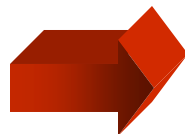
**Caracterización de una nueva *Pseudomonas* fluorescente responsable de causar manchas foliares en frijol en Mexico**



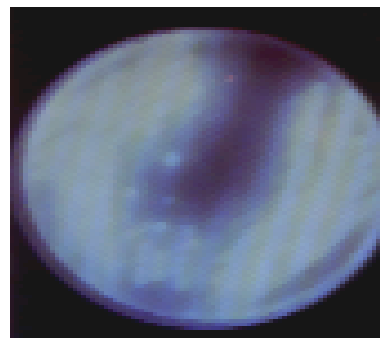
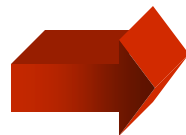


## Bacterias reportadas en el cultivo del frijol en México

**Tizón común**



**Tizón de halo**



## Bacterias reportadas en el cultivo del frijol en México

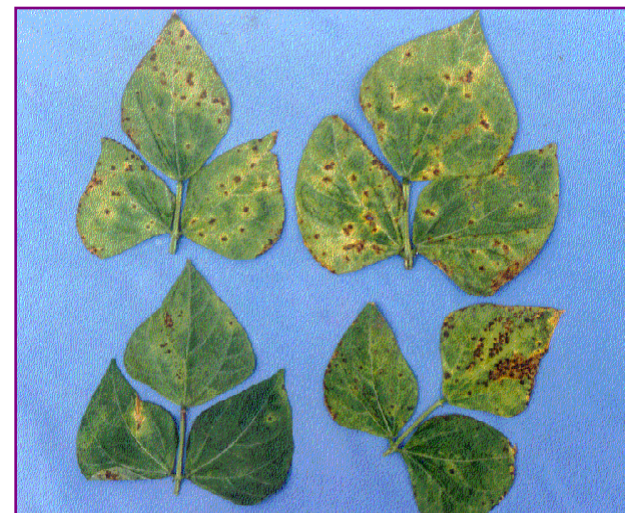
**Tizón común**

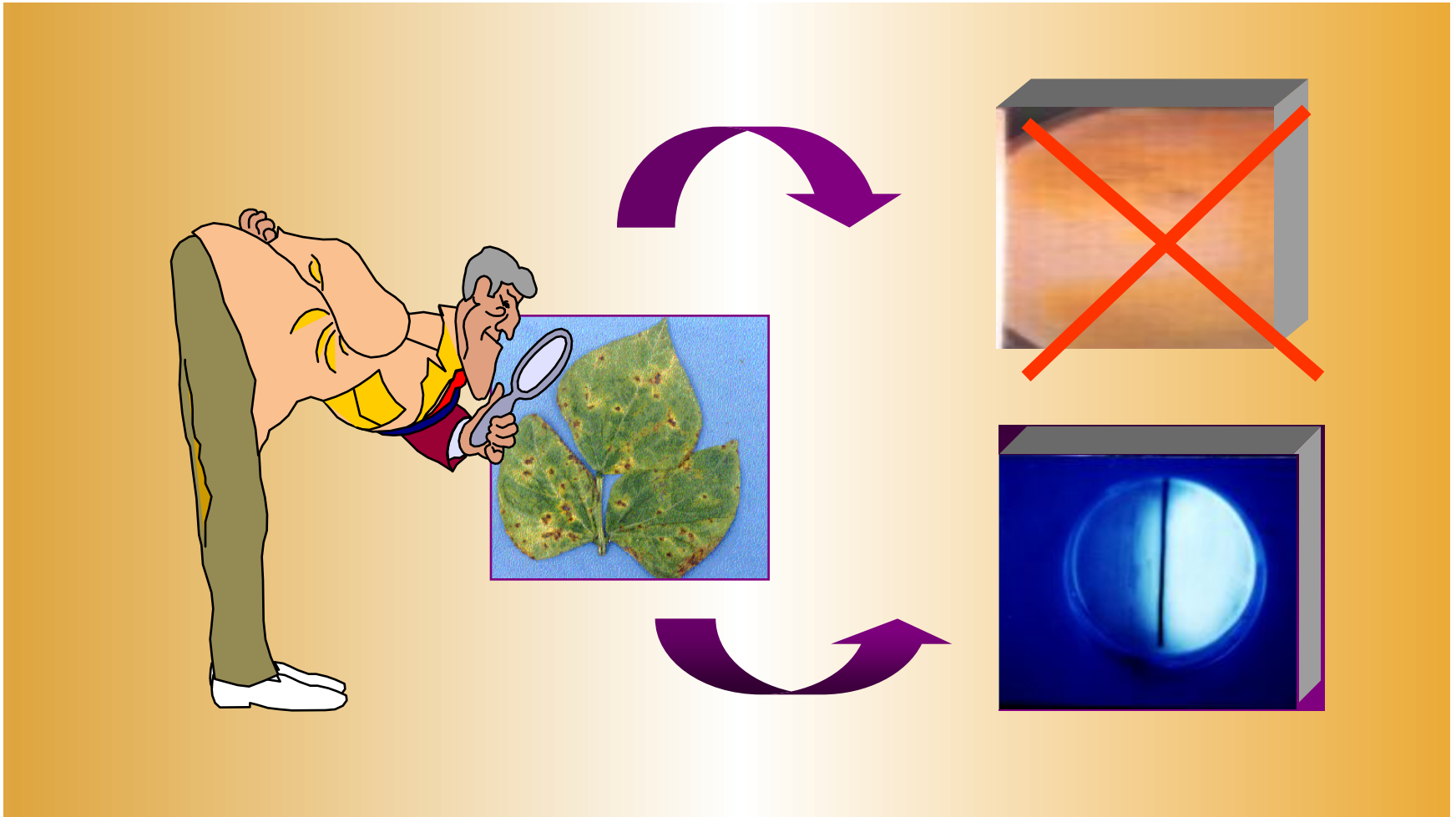


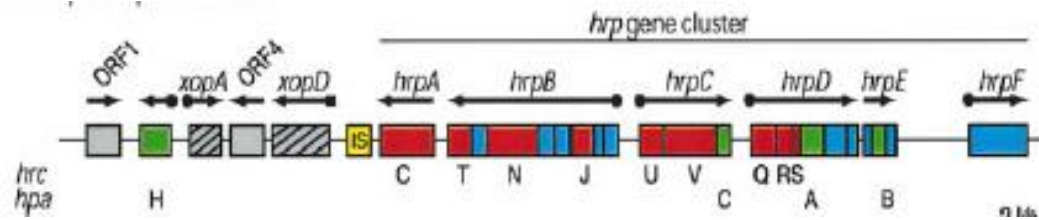
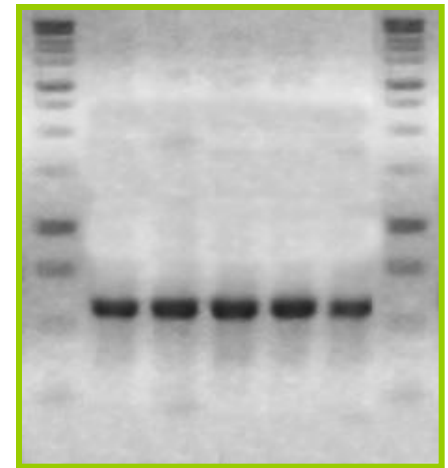
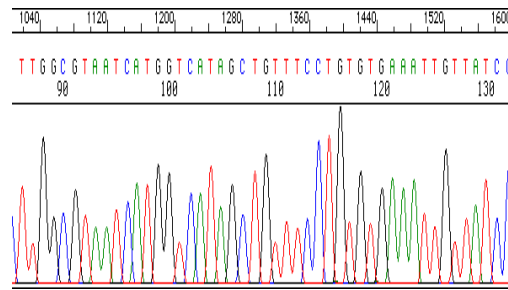
**Tizón de halo**



**¿Tizón?**







# Perfil del contenido de ácidos grasos de la membrana

El análisis se realizó en:

**National Collection Plant Pathogenic Bacteria**

**Central Science Laboratory**

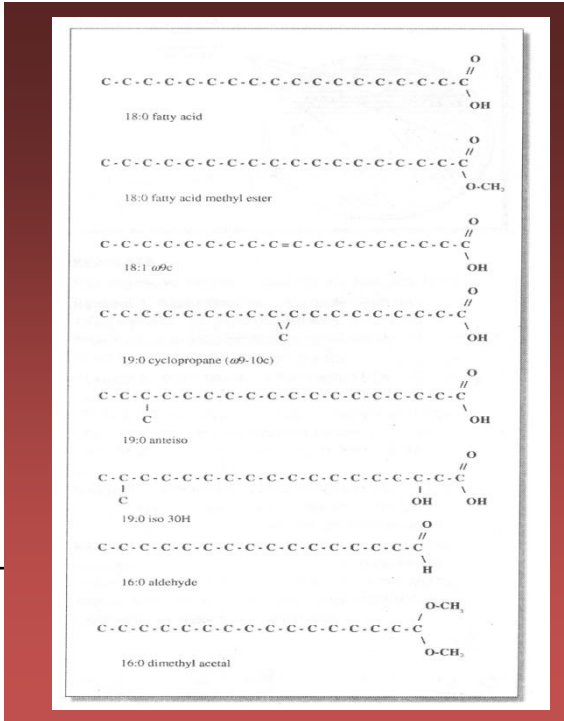
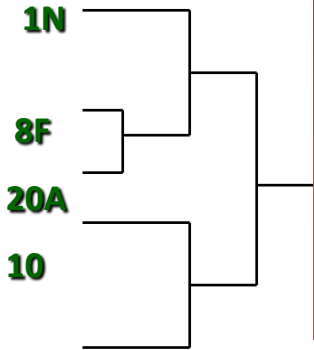
**Sand Hutton, York U.K.**

Las cepas se compararon con:

**Librería TSBA40**

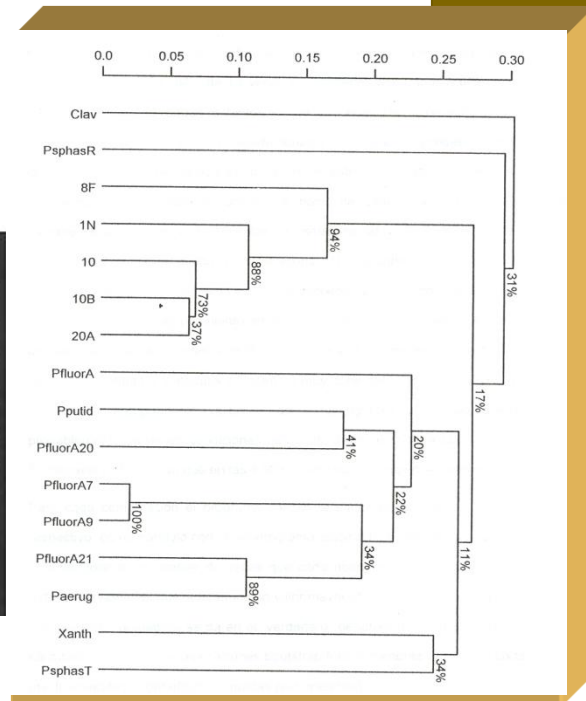
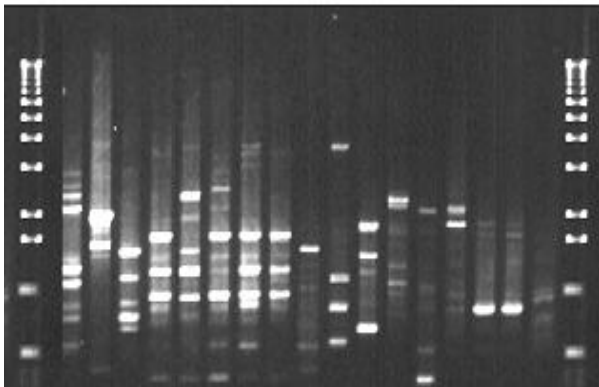
**NCPPB3**

**Especies no presentes en la librería**



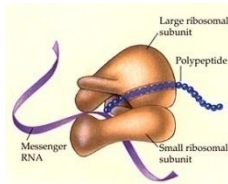
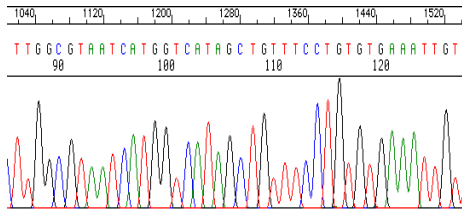






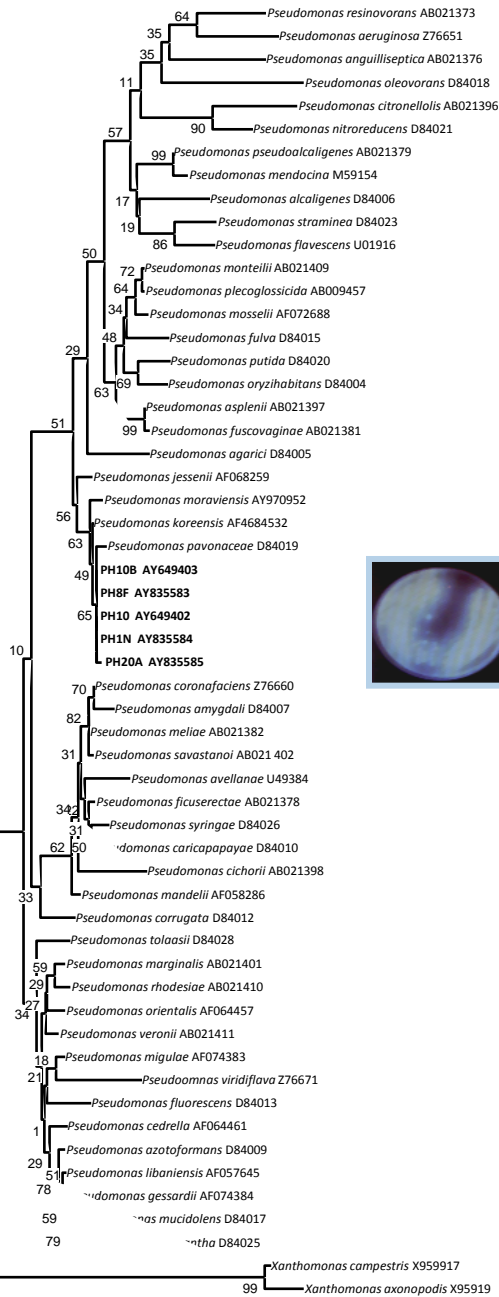
coeficiente de confianza de Felsenstein para cada agrupamiento (nodo) para lo cual se obtuvieron B=4000 repeticiones “bootstrap” (es decir, muestras aleatorias con reemplazo de las bandas), se usó el programa S-plus 4.5 (Mathsoft) para Windows.

*Pseudomonas* spp.  
In sensu stricto



16S rDNA

0.01



AERUGINOSA GROUP

PUTIDA GROUP

UNDEFINED GROUP

SYRINGAE GROUP

FLUORESCENS GROUP

# *Pantoea agglomerans* species complex, nuevo agente causal de estrias cloróticas en *Zea mays* y *Sorghum bicolor* en Mexico

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## plant disease

Editor-in-Chief: Anthony P. Keinath  
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October 2007, Volume 91, Number 10  
 Page 1365  
 DOI: 10.1094/PDIS-91-10-1365A

**Disease Notes**  
**First Report of *Pantoea agglomerans* Causing Leaf Blight and Vascular Wilt in Maize and Sorghum in Mexico**

**G. Morales-Valenzuela, H. V. Silva-Rojas, and D. Ochoa-Martínez,** Colegio de Postgraduados, Montecillo, Edo. de México, 56230, México; **E. Valdez-Moctezuma, B. Alarcón-Zúñiga,** and **L. X. Zelaya-Molina,** Universidad Autónoma Chapingo, Texcoco, Edo. de México, 56230, México; **L. Córdova-Téllez, L. Mendoza-Onofre, H. Vaquera-Huerta, and A. Carballo-Carballo,** Colegio de Postgraduados, Montecillo, Edo. de México, 56230, México; **A. Farfán-Gómez,** Universidad Autónoma Metropolitana-Xochimilco, D.F., 04960, México; and **G. Ávila-Quezada,** Centro de Investigación en Alimentación y Desarrollo-Delicias, Chihuahua, 33089, México

*Zea mays* and *Sorghum bicolor* are important crops for animal and human nutrition worldwide. In the Central Highland Valley of Mexico, both crops are extremely important, and research is aimed toward increasing yield, disease resistance, and crop adaptation from 1,900- to 2,700-m elevation. In a 3-year field breeding experiment (2004 to 2006), leaf blight and vascular wilt symptoms were frequently observed in contiguous plots of maize and sorghum crops in Montecillo, Mexico and maize plots in Tecamac, Mexico. To identify and characterize the causal agent of these symptoms, isolations were conducted on leaves from areas where healthy and diseased tissues converged. Leaf sections of 1 cm<sup>2</sup> from both crops were disinfested, placed on caseinamino acid-peptone-glucose (CPC)

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## AISLAMIENTO DE HONGOS

### GRANOS ASINTOMÁTICOS

*Fusarium verticillioides*

Grupo de compatibilidad A

*Fusarium subglutinans*

Grupo de compatibilidad B

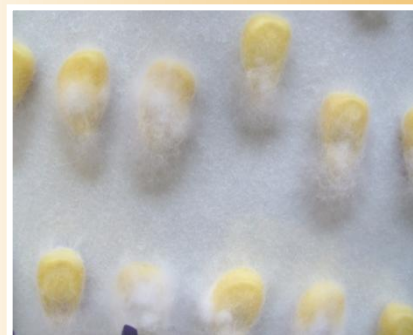
*Fusarium proliferatum*



0 DÍAS



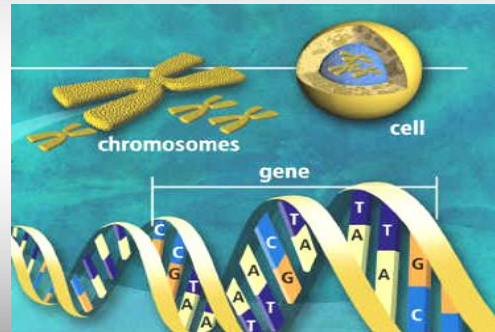
4 DÍAS



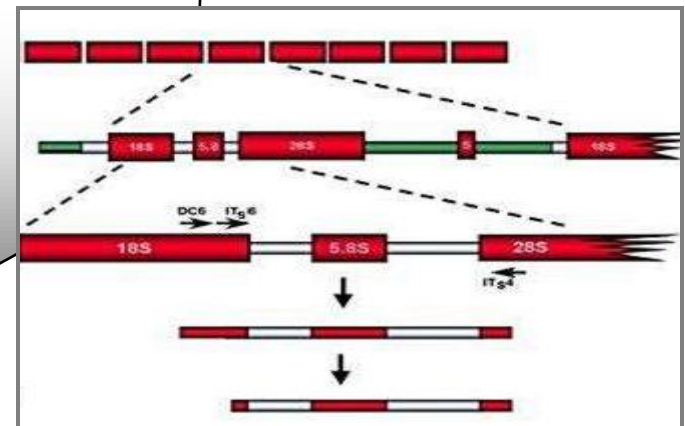
8 DÍAS



# Genes nucleares



## Organización del ribosoma



## Región del ITS

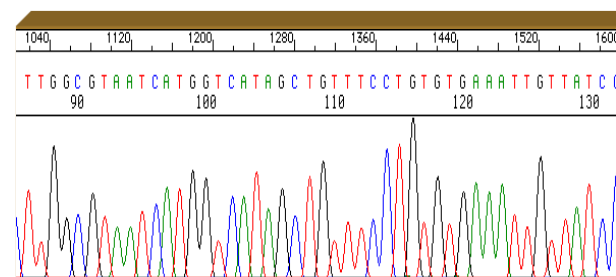
# Espece Filogenética

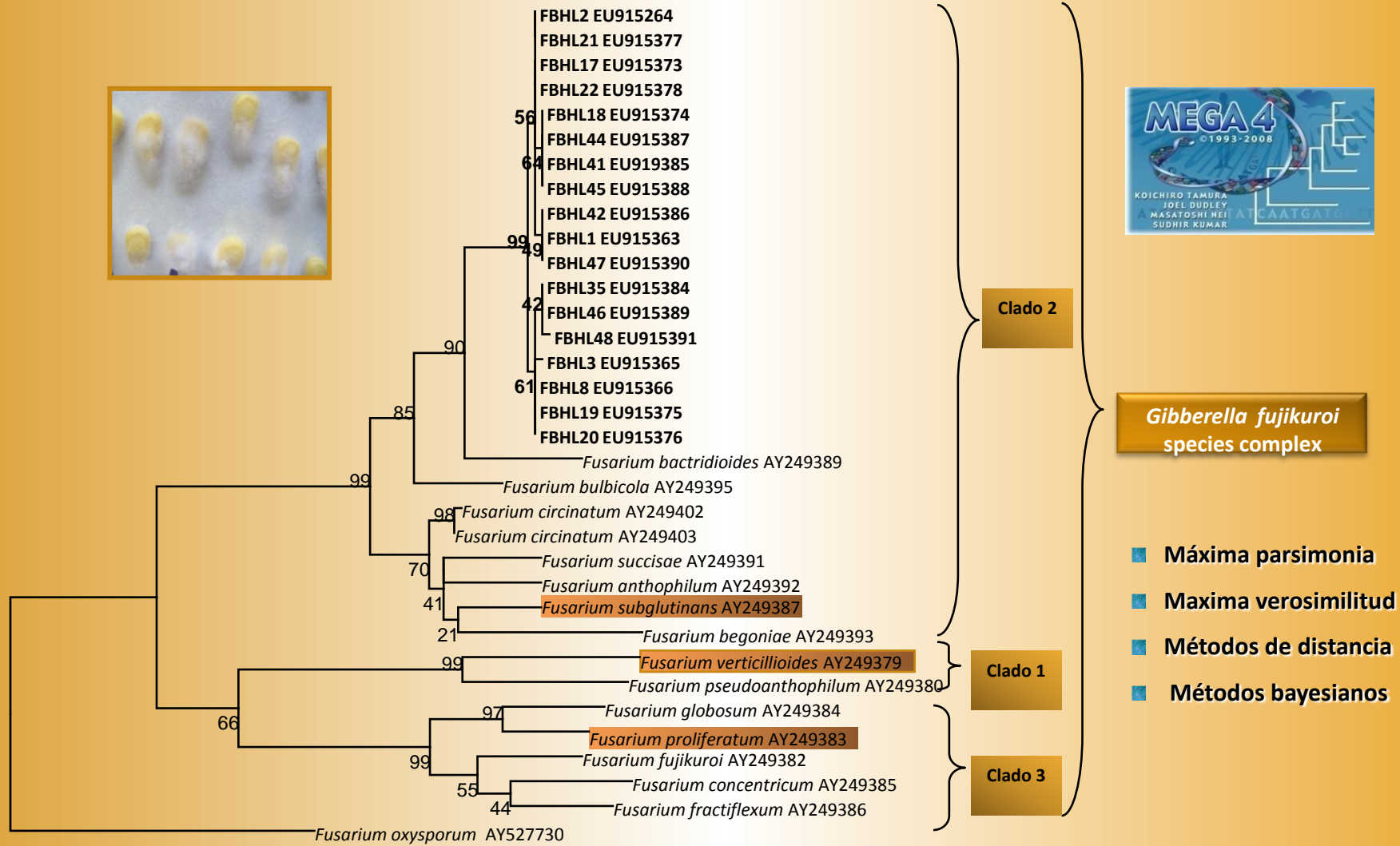
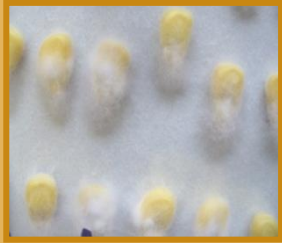
## Definición de especie por

- Hibridación DNA-DNA
- Subunidad pequeña del rRNA
- MultiLocus Sequences Typing

**MLST**

Amplificación de genes de 1 o muy pocas copias  
Genes Housekeeping



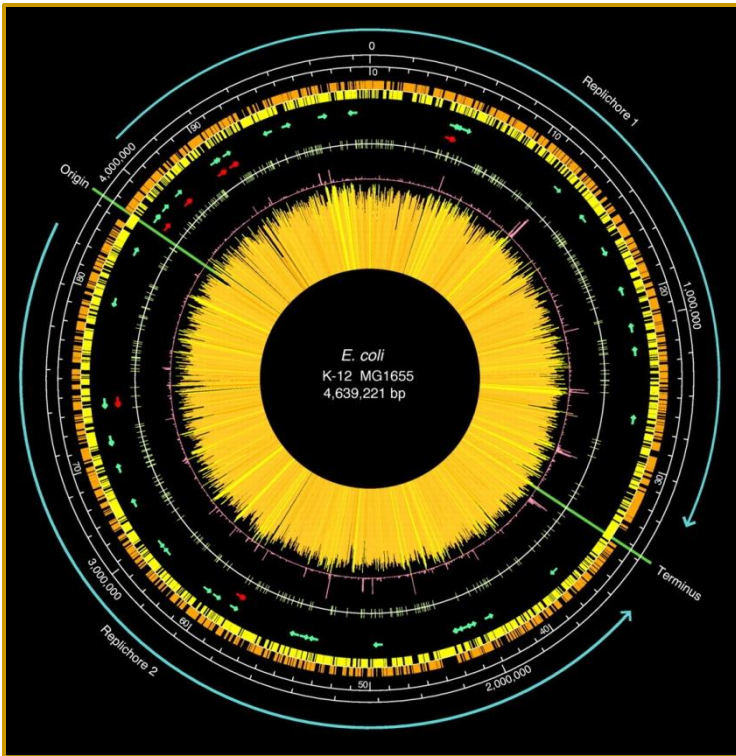


***Gibberella fujikuroi* species complex**

- Máxima parsimonia
- Maxima verosimilitud
- Métodos de distancia
- Métodos bayesianos

10





**G. Jamjoom 2005**

## Secuenciación de genomas completos

*Pantoea agglomerans*  
*Pantoea ananatis*  
*Pantoea stewartii*  
*Pseudomonas montecillense* sp. nova







**Muchas gracias**

