Genetics and genomics of disease resistance in pepper: focus on Phytophthora threats

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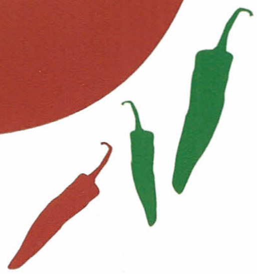
Véronique Lefebvre

International Symposium
Commemorating
the 20th Anniversary of
Korea Capsicum Research Cooperative

Pepper Diseases and Resistance Breeding



Date | **September 22~23, 2011**
Place | **Gaeshin Culture Center,**
Chungbuk National University, Korea

Organizers

Korea Capsicum Research Cooperative
National Institute of Horticultural and Herbal Science, RDA
Vegetable Breeding Research Center, SNU

Coorganizers

Center for Pepper Molecular Markers
Screening Center for Disease Resistant Vegetable Crops
Molecular & Analytical Service Center for Crop Improvement
Pepper Consortium in Chungbuk
AVRDC – The World Vegetable Center

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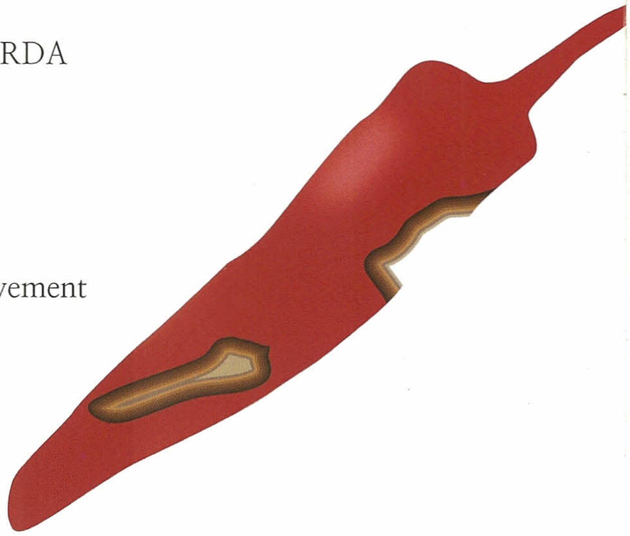

Website _ www.capsicum.or.kr

S2-4

Genetics and genomics of disease resistance in pepper: focus on *Phytophthora threats*

Véronique Lefebvre*

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Because of its widespread geographical distribution throughout the world from the intertropical belt to northern latitudes, cultivated peppers (*Capsicum annuum*) are vulnerable to many pathogens. The virus complexes and the Oomycete *Phytophthora* cause the major damages. *Phytophthora* blight remains one of the most serious problems worldwide, even if management practices help to control epidemics. *P. capsici* is predominant; *P. parasitica* (=*P. nicotianae*) was sporadically reported. No major resistance gene to *Phytophthora* was deployed in cultivars; only a few exotic accessions with partial polygenic resistances were reported. Ongoing genetic, genomic and histo-cytological analyses aim to unravel genetic, molecular and functional bases of those reported resistances.

The quantitative assessment of resistance to *Phytophthora* in several mapping populations and its dissection into quantitative trait loci (QTLs) highlighted its complex genetic architecture and its diversity. Identified QTLs appeared either specific to *P. capsici* or to *P. parasitica*. According to the resistant parent and the mapping population analysed, between 4 and 12 resistance QTLs to *P. capsici* were detected on the whole genome. QTLs display additive and/or epistatic effects.

Phenotypic and molecular analyses of populations from phenotypic selection for *P. capsici* resistance pointed out that low effect resistance alleles and epistatic interactions were lost after several backcrosses, explaining difficulties to transfer this resistance from exotic resistant accessions to elite cultivars. Conversely, when achieving marker-assisted backcrosses, four QTLs with additive and epistatic effects were validated, and elite resistant lines obtained.

QTL studies from our lab and literature have systematically identified resistance QTLs to *P. capsici* on pepper chromosome P5. These P5 QTLs have a major effect on resistance to *P. capsici*. After adding anchor markers from tomato and pepper literature and performing a comparative analysis by fine mapping and meta-analysis, we highlighted a QTL cluster involved in *P. capsici* resistance on chromosome P5. To further investigate this major effect resistance QTL cluster on P5, near-isogenic lines were constructed. They are used in fine mapping and for transcriptomic analyses.

A diversity analysis of *P. capsici* isolates collected in South of France revealed both mating types and a high number of SNP haplotypes in a same sampled field, suggesting the presence of sexual populations and a rapid evolution of *P. capsici* population. To investigate the potential durability of major effect QTLs on P5, near-isogenic lines containing or not resistance alleles at P5 QTLs in a susceptible background were tested with four *P. capsici* isolates that we determined to be genetically different. Our results showed that the major effect QTL cluster on P5 is effective against the four tested isolates. Moreover, comparative mapping with published QTLs on P5 suggests that the major effect QTL on P5 is common to 5 resistant lines, and it confers a broad spectrum resistance to the 8 *P. capsici* isolates from different geographical origins, tested so far.

To investigate the functional bases of pepper / *P. capsici* interaction, we developed an efficient *Agrobacterium rhizogenes*-mediated transformation system for producing hairy roots of *C. annuum*. We showed by histo-cytological analysis that transformed and untransformed roots displayed the same resistance response to *P. capsici*. In a susceptible line, cells of elongation zone of early infected roots are severely disorganised and invaded by mycelium, while in the resistant line 'Criollo de Morelos 334', a necrotic reaction of the root epidermis stops the parasite.

Our results, together with ongoing sequencing programs in Solanaceae and *Phytophthora*, are expected to increase the knowledge on the Solanaceae-*Phytophthora* crosstalk.

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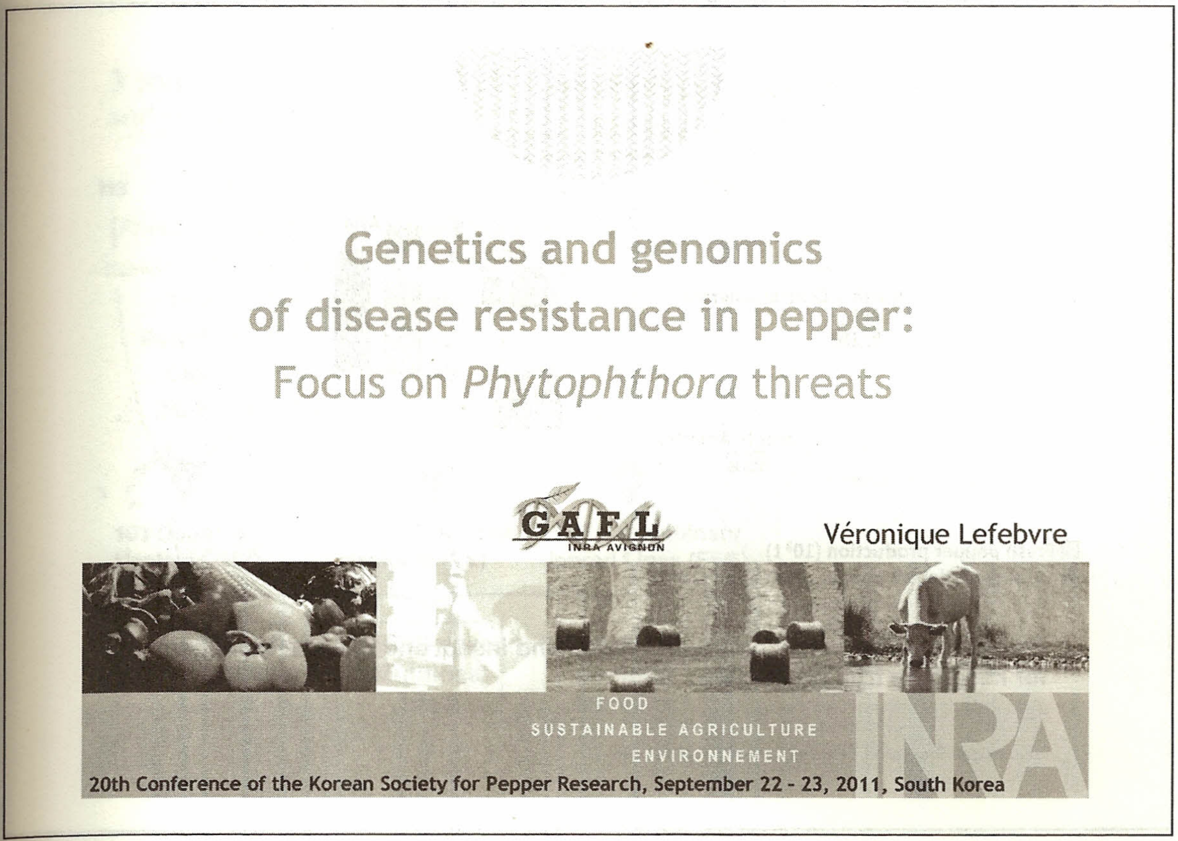


Genetics and genomics of disease resistance in pepper:

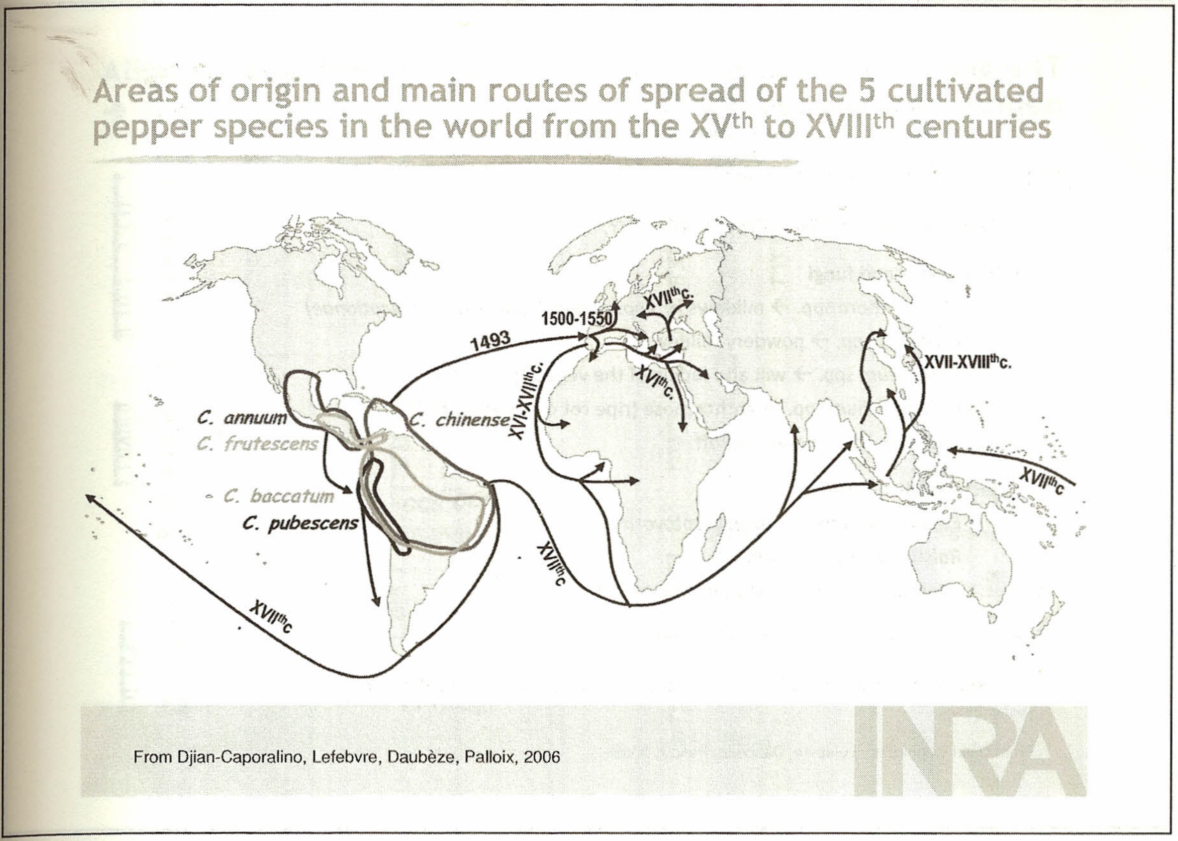
Focus on *Phytophthora* threats

GAFL INRA AVIGNON

Véronique Lefebvre

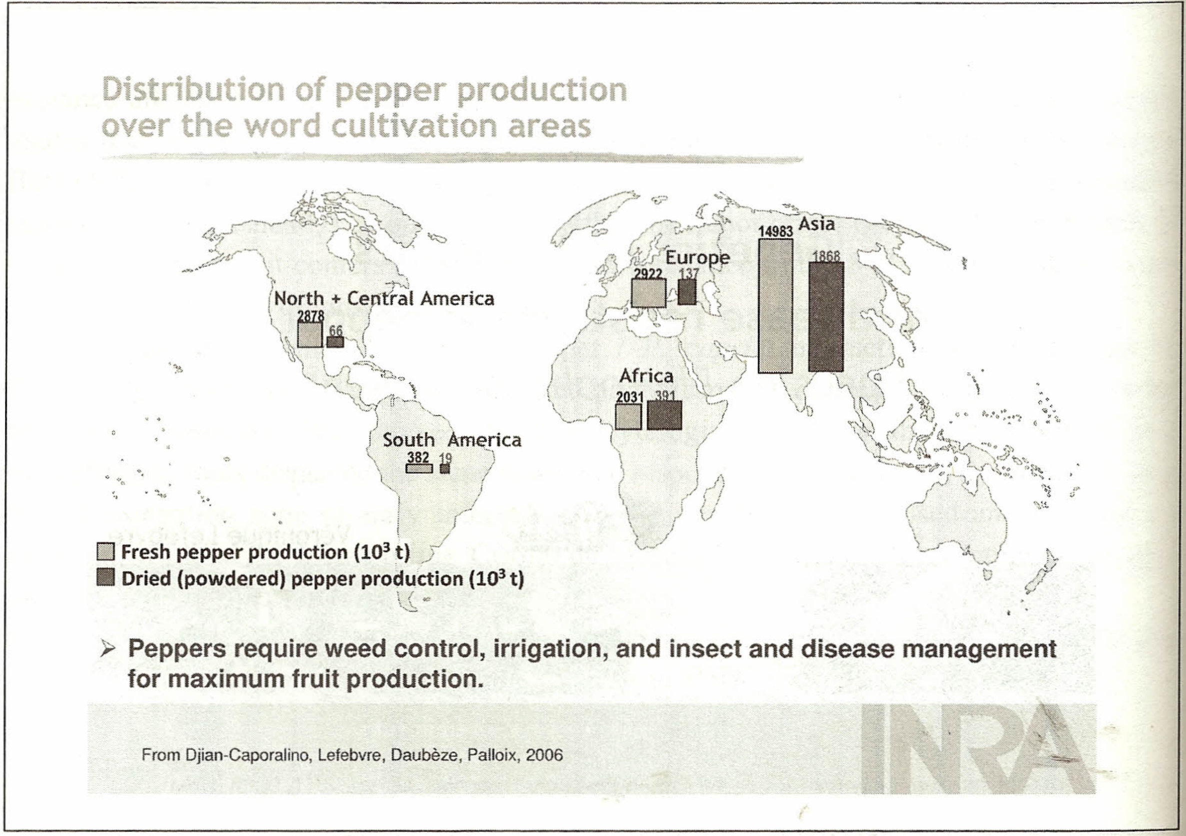
FOOD
SUSTAINABLE AGRICULTURE
ENVIRONNEMENT

20th Conference of the Korean Society for Pepper Research, September 22 - 23, 2011, South Korea



Areas of origin and main routes of spread of the 5 cultivated pepper species in the world from the XVth to XVIIIth centuries

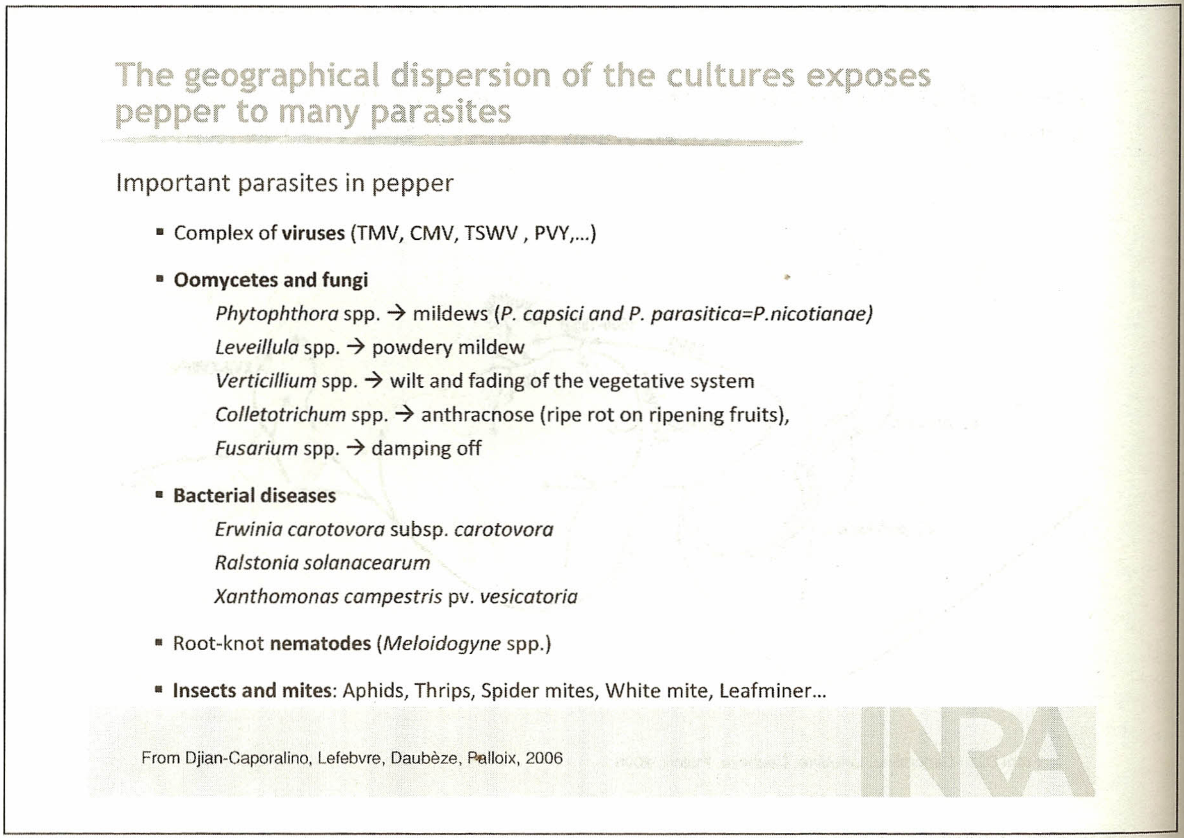
From Djian-Caporalino, Lefebvre, Daubèze, Palloix, 2006



Distribution of pepper production over the word cultivation areas

➢ **Peppers require weed control, irrigation, and insect and disease management for maximum fruit production.**

From Djian-Caporalino, Lefebvre, Daubèze, Palloix, 2006

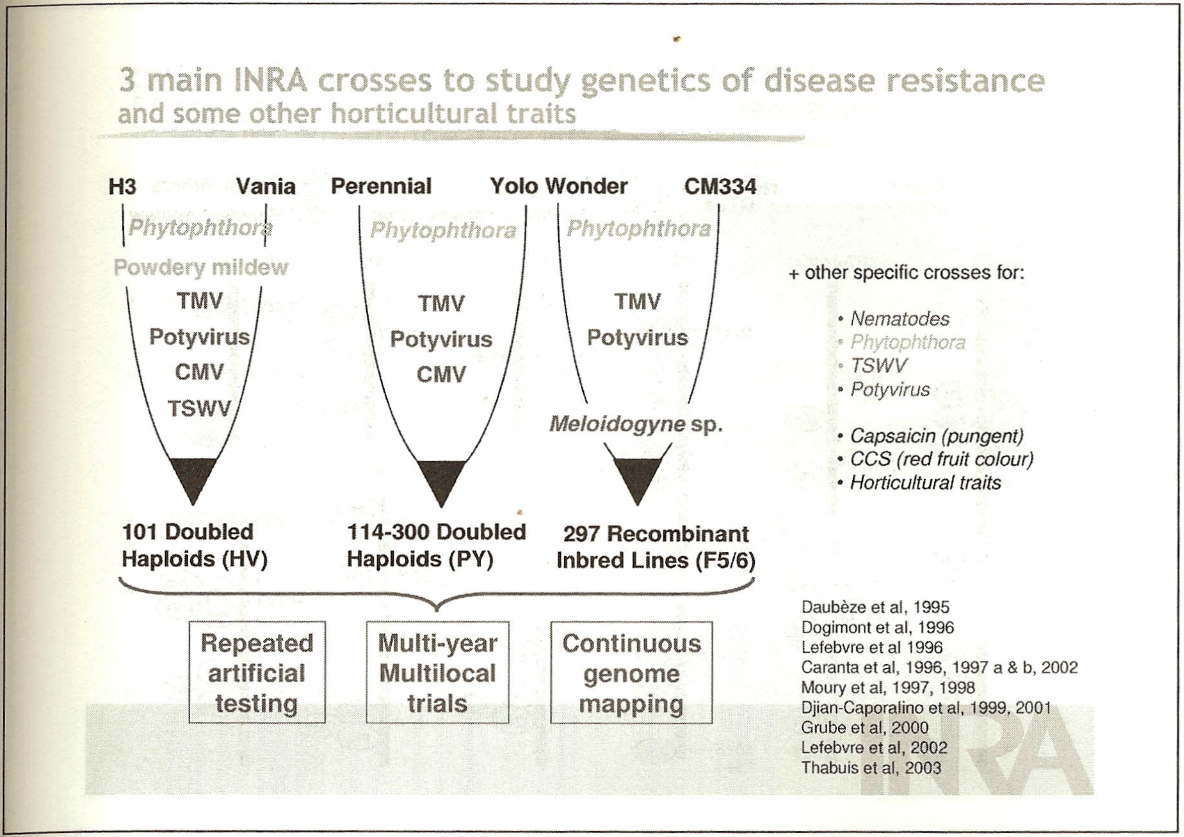


The geographical dispersion of the cultures exposes pepper to many parasites

Important parasites in pepper

- Complex of **viruses** (TMV, CMV, TSWV , PVY,...)
- **Oomycetes and fungi**
  Phytophthora spp. → mildews (*P. capsici and P. parasitica=P.nicotianae)*
  Leveillula spp. → powdery mildew
  Verticillium spp. → wilt and fading of the vegetative system
  Colletotrichum spp. → anthracnose (ripe rot on ripening fruits),
  Fusarium spp. → damping off
- **Bacterial diseases**
  Erwinia carotovora subsp. *carotovora*
  Ralstonia solanacearum
  Xanthomonas campestris pv. *vesicatoria*
- Root-knot **nematodes** (*Meloidogyne* spp.)
- **Insects and mites**: Aphids, Thrips, Spider mites, White mite, Leafminer...

From Djian-Caporalino, Lefebvre, Daubèze, Palloix, 2006



3 main INRA crosses to study genetics of disease resistance
and some other horticultural traits

H3 × **Vania**: *Phytophthora*, Powdery mildew, TMV, Potyvirus, CMV, TSWV → **101 Doubled Haploids (HV)**

Perennial × **Yolo Wonder**: *Phytophthora*, TMV, Potyvirus, CMV → **114-300 Doubled Haploids (PY)**

Yolo Wonder × **CM334**: *Phytophthora*, TMV, Potyvirus, *Meloidogyne* sp. → **297 Recombinant Inbred Lines (F5/6)**

Repeated artificial testing

Multi-year Multilocal trials

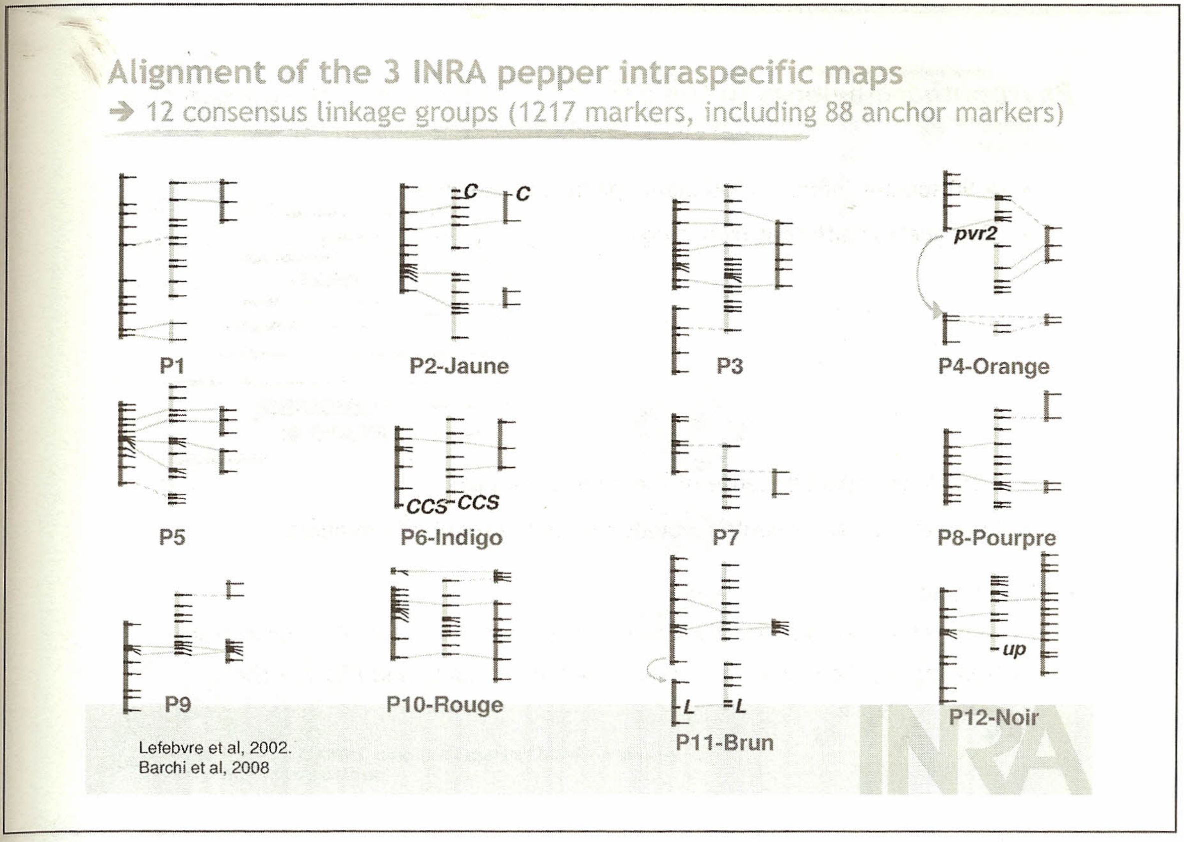
Continuous genome mapping

+ other specific crosses for:

- *Nematodes*
- *Phytophthora*
- *TSWV*
- *Potyvirus*

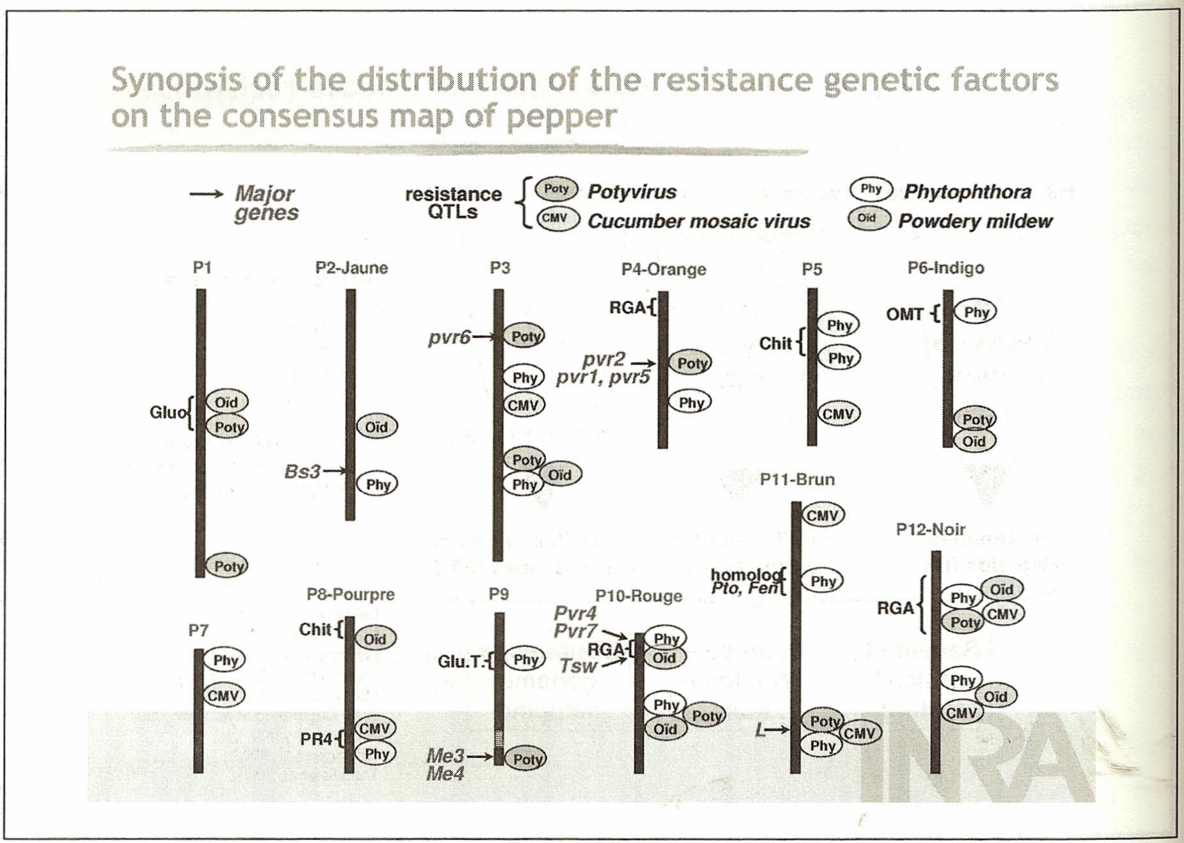
- *Capsaicin (pungent)*
- *CCS (red fruit colour)*
- *Horticultural traits*

Daubèze et al, 1995
Dogimont et al, 1996
Lefebvre et al 1996
Caranta et al, 1996, 1997 a & b, 2002
Moury et al, 1997, 1998
Djian-Caporalino et al, 1999, 2001
Grube et al, 2000
Lefebvre et al, 2002
Thabuis et al, 2003

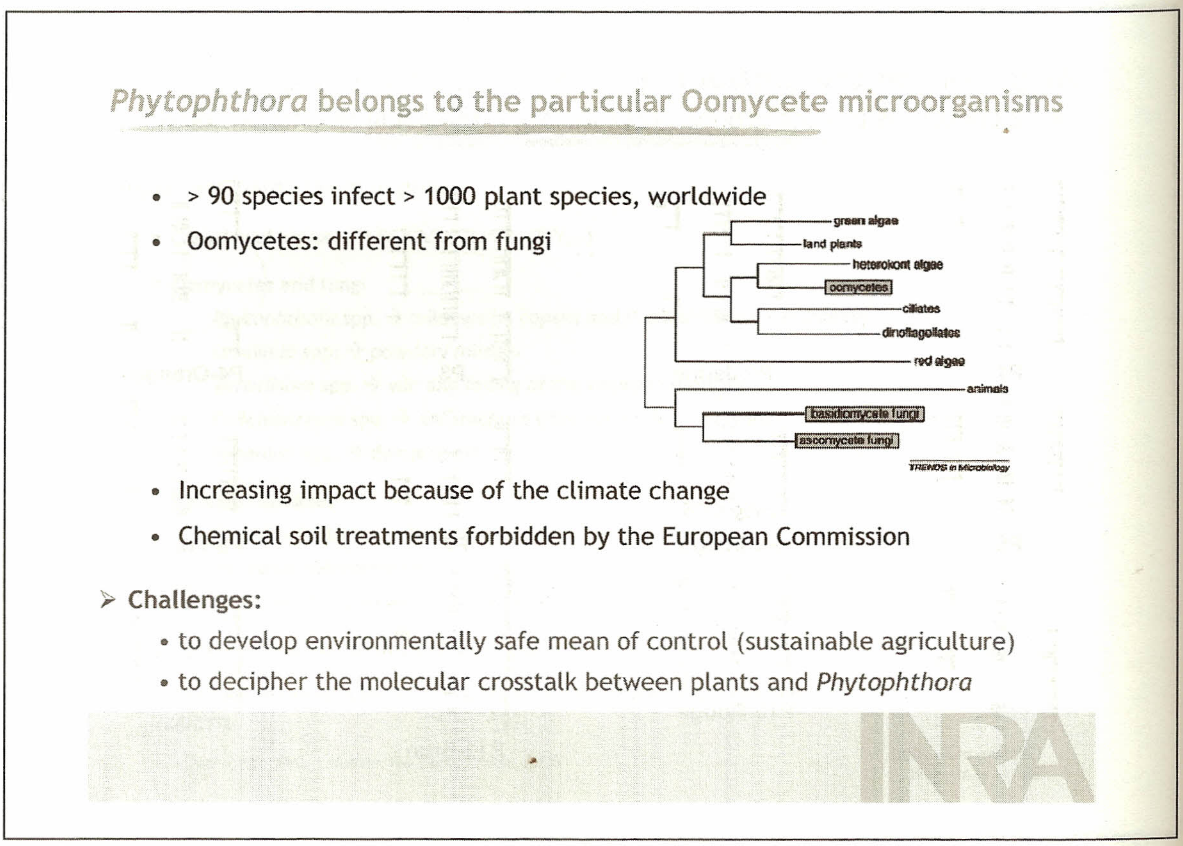


Alignment of the 3 INRA pepper intraspecific maps
➔ 12 consensus linkage groups (1217 markers, including 88 anchor markers)

Lefebvre et al, 2002.
Barchi et al, 2008



Synopsis of the distribution of the resistance genetic factors on the consensus map of pepper

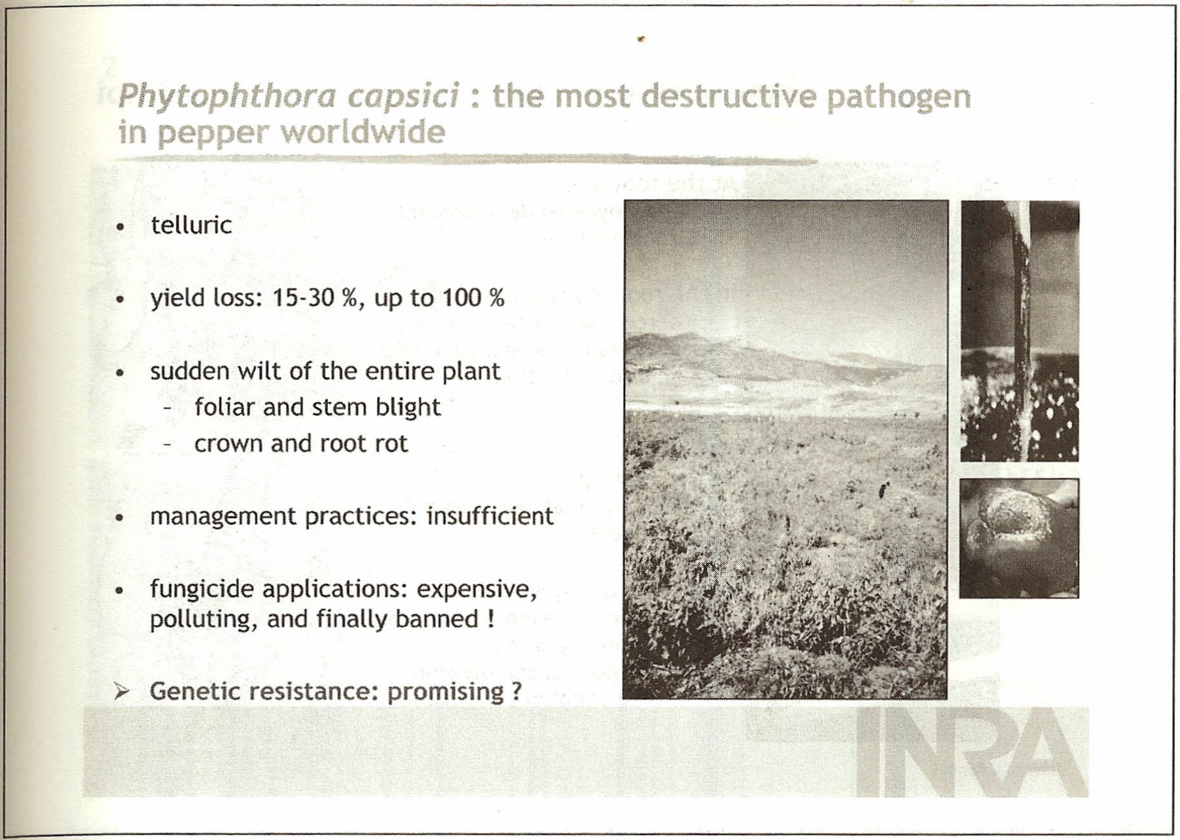


Phytophthora belongs to the particular Oomycete microorganisms

- > 90 species infect > 1000 plant species, worldwide
- Oomycetes: different from fungi

- Increasing impact because of the climate change
- Chemical soil treatments forbidden by the European Commission

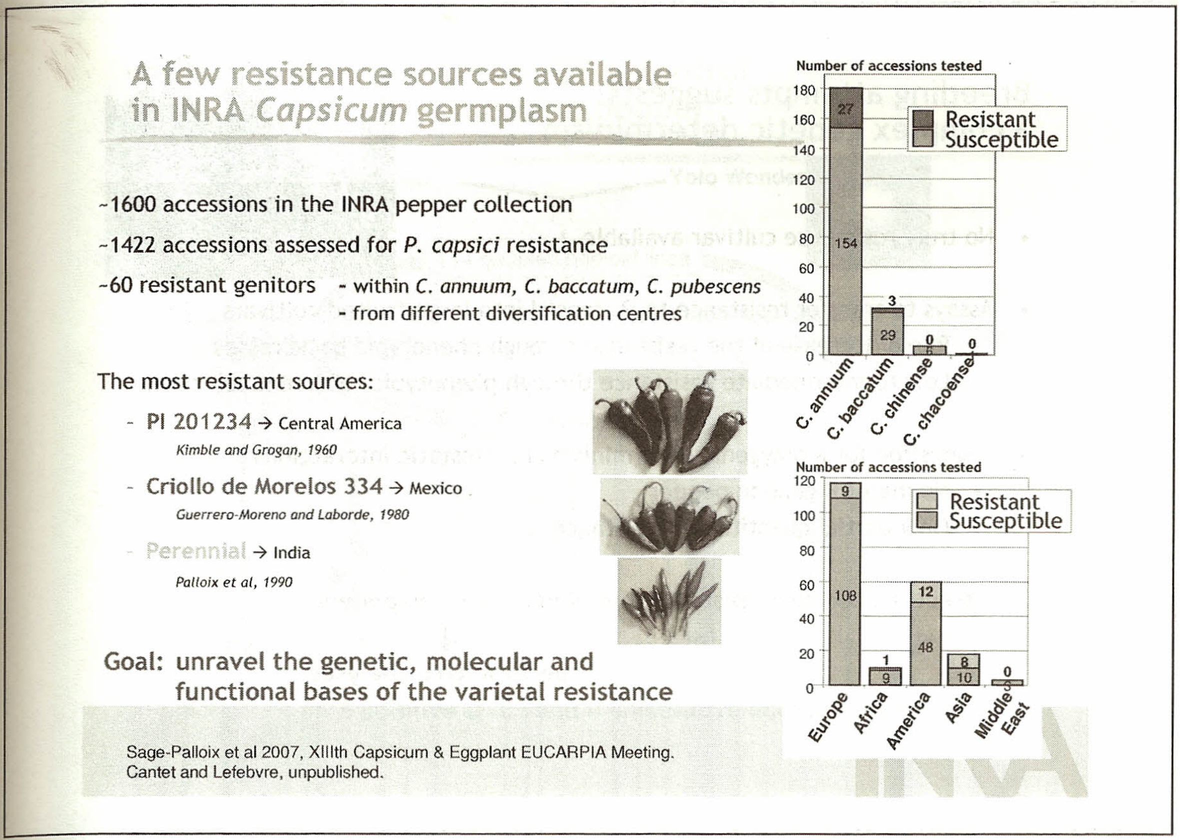
➢ **Challenges:**
  - to develop environmentally safe mean of control (sustainable agriculture)
  - to decipher the molecular crosstalk between plants and *Phytophthora*



Phytophthora capsici : the most destructive pathogen in pepper worldwide

- telluric
- yield loss: 15-30 %, up to 100 %
- sudden wilt of the entire plant
  - foliar and stem blight
  - crown and root rot
- management practices: insufficient
- fungicide applications: expensive, polluting, and finally banned !

➢ Genetic resistance: promising ?



A few resistance sources available in INRA *Capsicum* germplasm

~1600 accessions in the INRA pepper collection

~1422 accessions assessed for *P. capsici* resistance

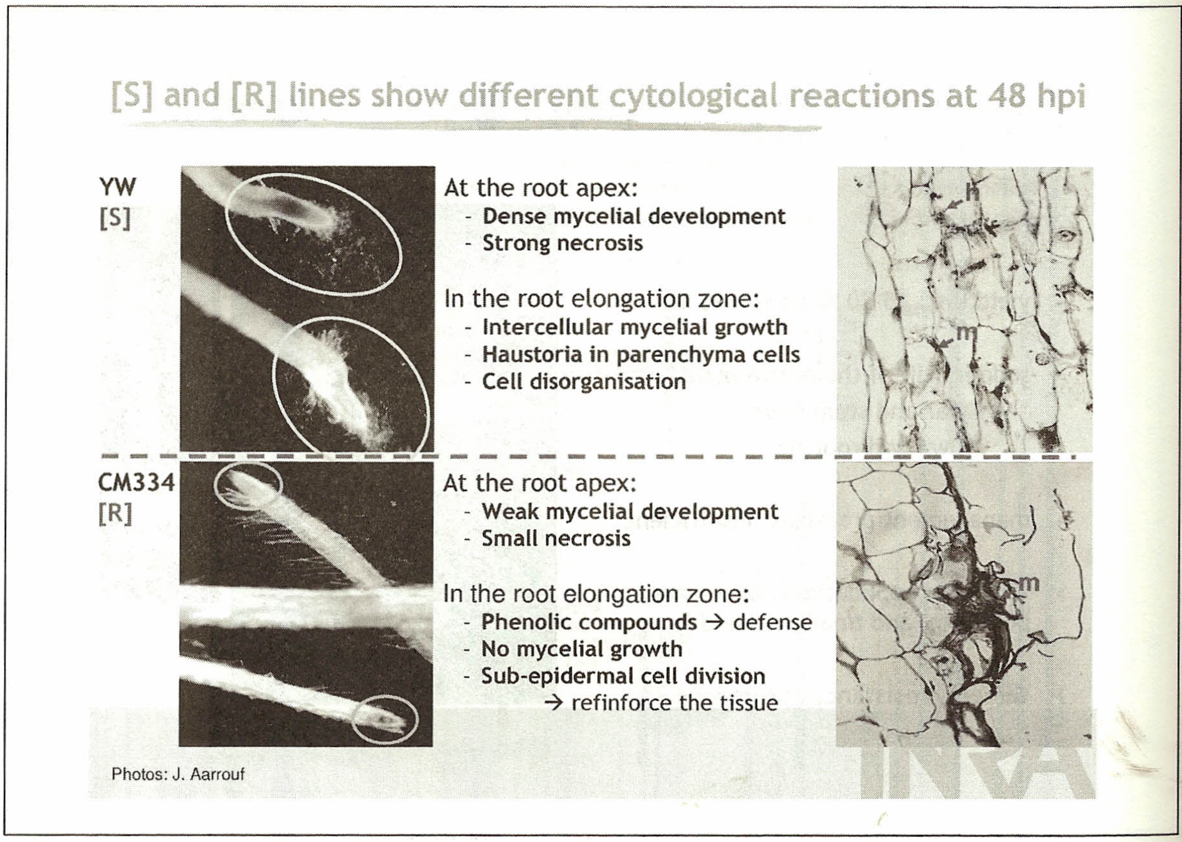
~60 resistant genitors - within *C. annuum, C. baccatum, C. pubescens*
- from different diversification centres

The most resistant sources:

- PI 201234 → Central America
  Kimble and Grogan, 1960
- Criollo de Morelos 334 → Mexico
  Guerrero-Moreno and Laborde, 1980
- Perennial → India
  Palloix et al, 1990

Goal: unravel the genetic, molecular and functional bases of the varietal resistance

Sage-Palloix et al 2007, XIIIth Capsicum & Eggplant EUCARPIA Meeting.
Cantet and Lefebvre, unpublished.



[S] and [R] lines show different cytological reactions at 48 hpi

YW
[S]

At the root apex:
- **Dense mycelial development**
- **Strong necrosis**

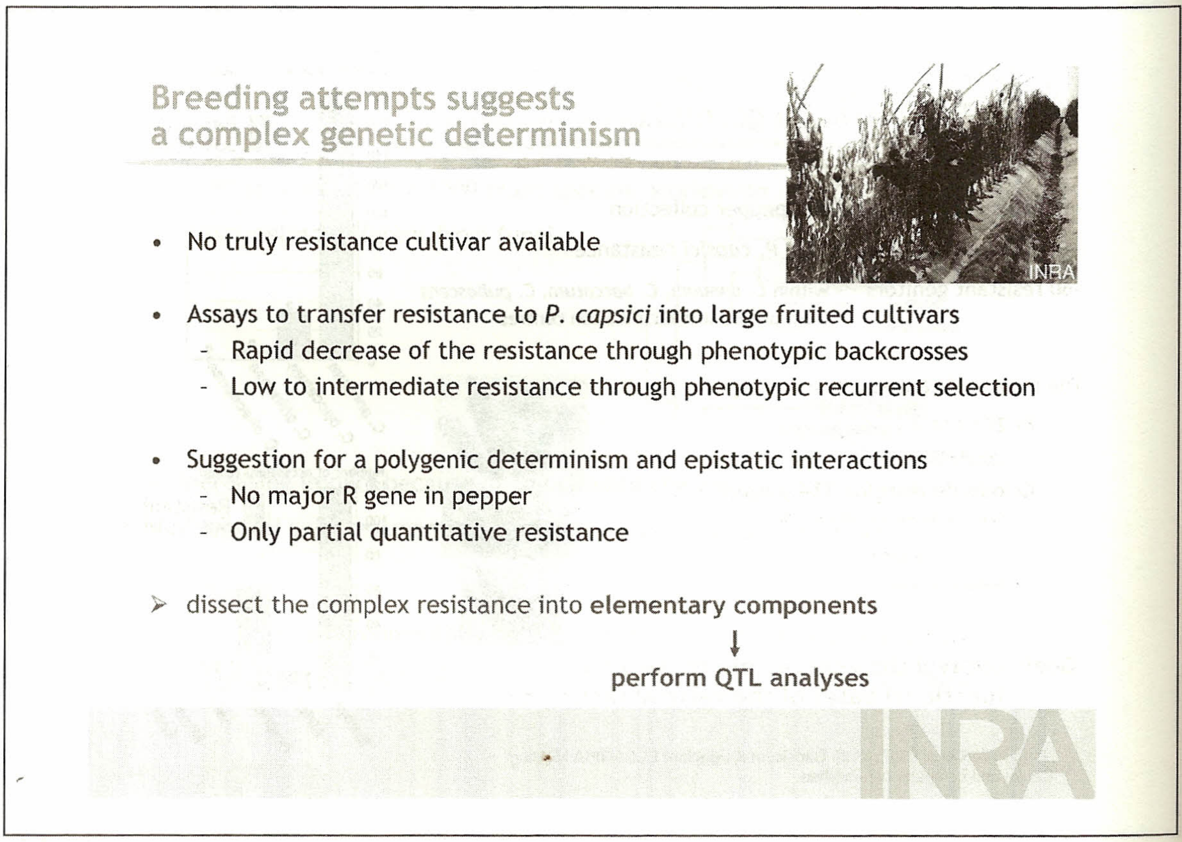
In the root elongation zone:
- **Intercellular mycelial growth**
- **Haustoria in parenchyma cells**
- **Cell disorganisation**

CM334
[R]

At the root apex:
- **Weak mycelial development**
- **Small necrosis**

In the root elongation zone:
- **Phenolic compounds** → defense
- **No mycelial growth**
- **Sub-epidermal cell division**
  → refinforce the tissue

Photos: J. Aarrouf



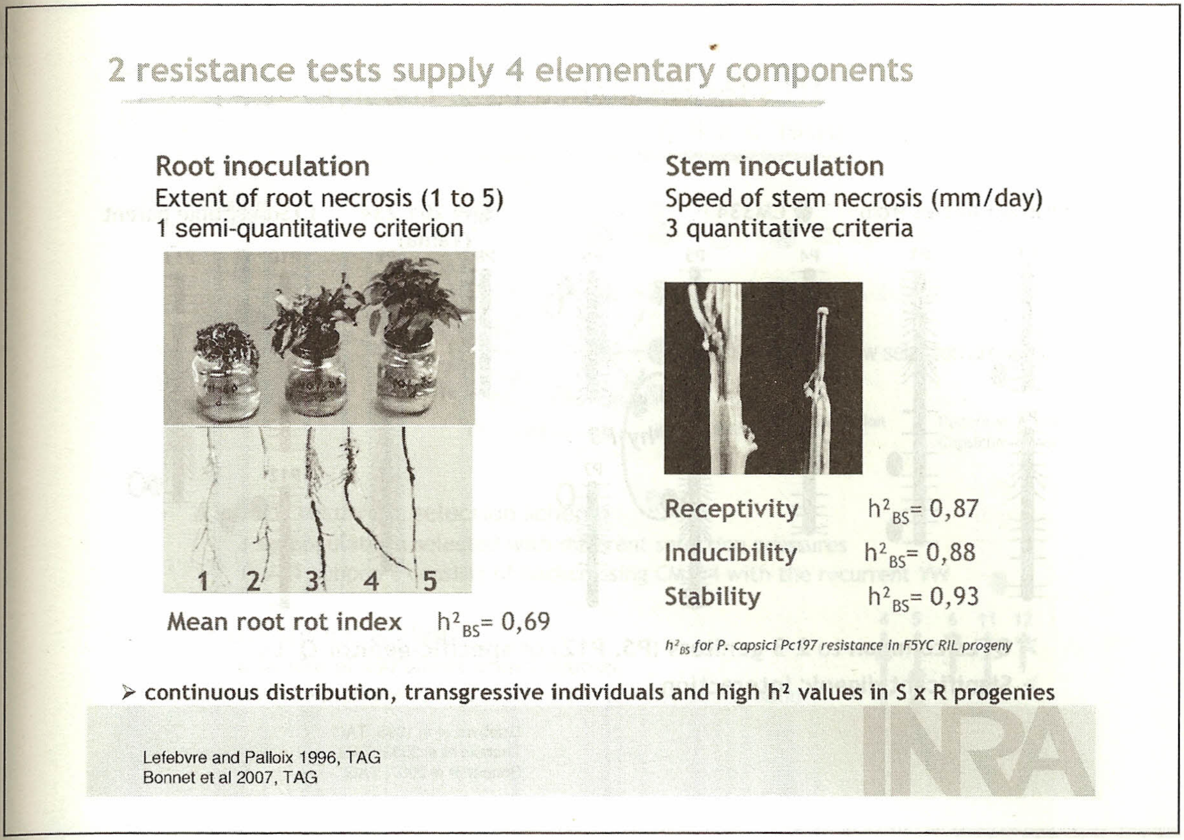
Breeding attempts suggests a complex genetic determinism

- No truly resistance cultivar available
- Assays to transfer resistance to *P. capsici* into large fruited cultivars
  - Rapid decrease of the resistance through phenotypic backcrosses
  - Low to intermediate resistance through phenotypic recurrent selection
- Suggestion for a polygenic determinism and epistatic interactions
  - No major R gene in pepper
  - Only partial quantitative resistance

➢ dissect the complex resistance into **elementary components**

↓

perform QTL analyses



2 resistance tests supply 4 elementary components

Root inoculation
Extent of root necrosis (1 to 5)
1 semi-quantitative criterion

Mean root rot index h^2_{BS}= 0,69

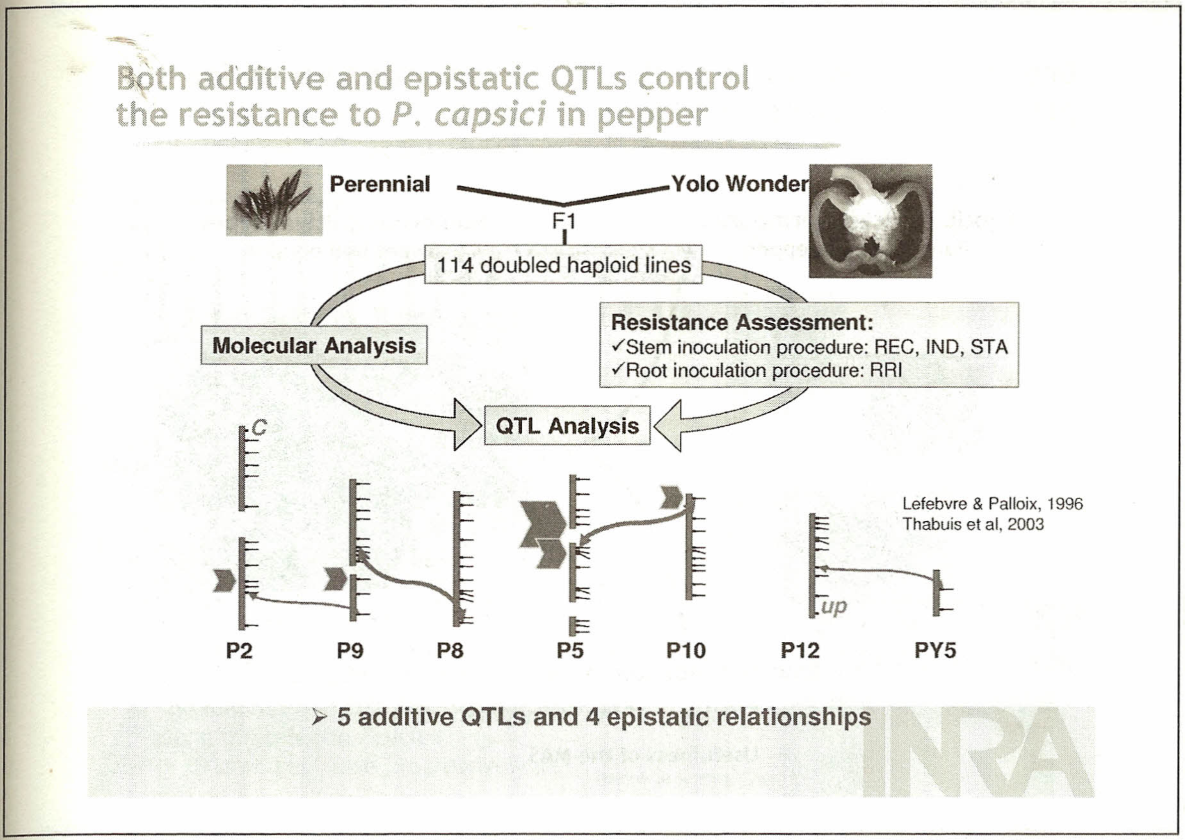
Stem inoculation
Speed of stem necrosis (mm/day)
3 quantitative criteria

Receptivity	h^2_{BS}= 0,87
Inducibility	h^2_{BS}= 0,88
Stability	h^2_{BS}= 0,93

h^2_{BS} for P. capsici Pc197 resistance in F5YC RIL progeny

➢ continuous distribution, transgressive individuals and high h^2 values in S x R progenies

Lefebvre and Palloix 1996, TAG
Bonnet et al 2007, TAG

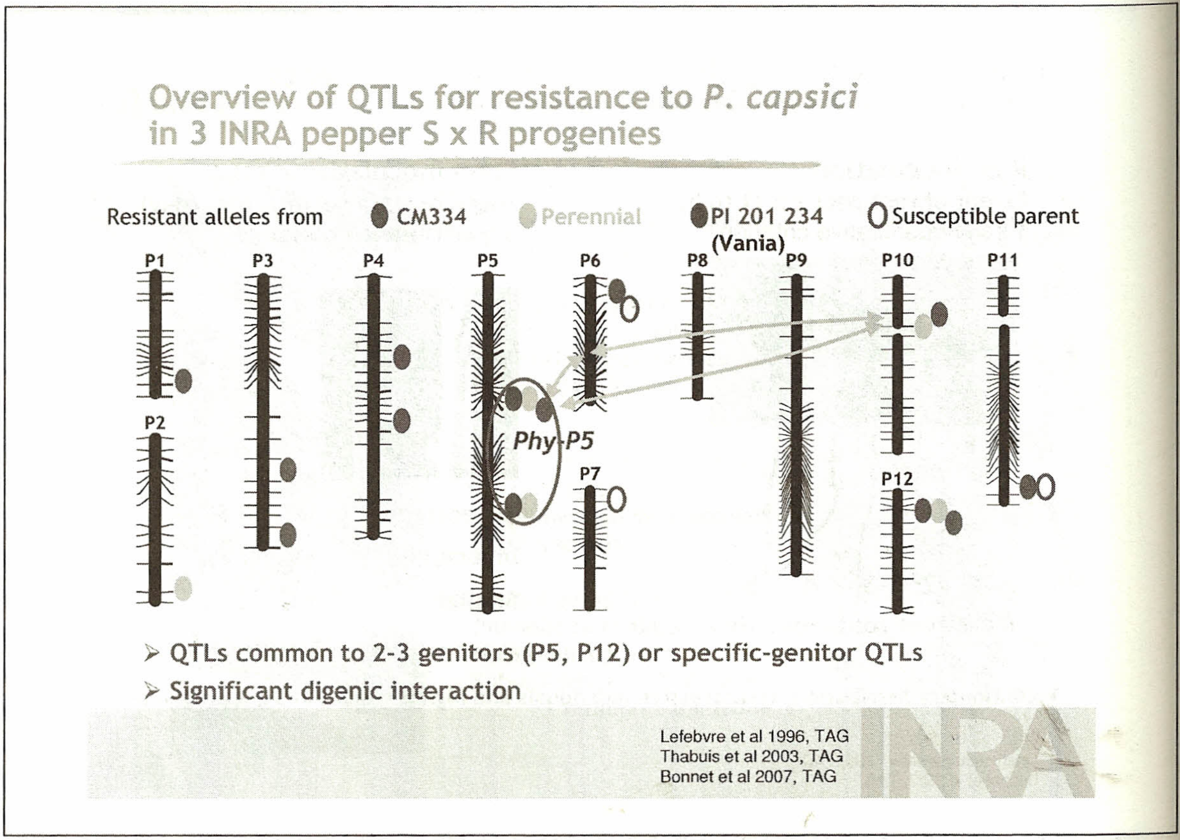


Both additive and epistatic QTLs control the resistance to *P. capsici* in pepper

Perennial
Yolo Wonder
F1
114 doubled haploid lines
Molecular Analysis
Resistance Assessment:
✓Stem inoculation procedure: REC, IND, STA
✓Root inoculation procedure: RRI
QTL Analysis

Lefebvre & Palloix, 1996
Thabuis et al, 2003

➢ **5 additive QTLs and 4 epistatic relationships**

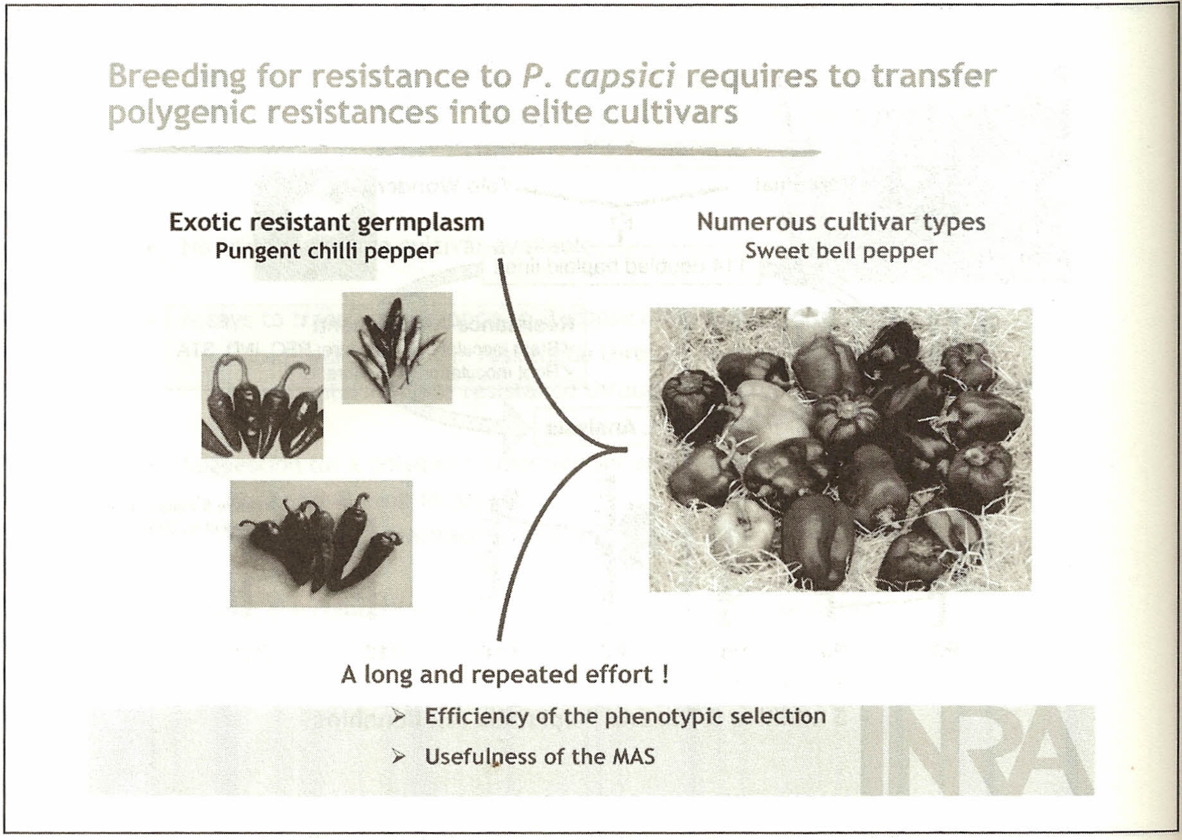


Overview of QTLs for resistance to *P. capsici* in 3 INRA pepper S x R progenies

Resistant alleles from ● CM334 ● Perennial ● PI 201 234 (Vania) ○ Susceptible parent

- ➢ QTLs common to 2-3 genitors (P5, P12) or specific-genitor QTLs
- ➢ Significant digenic interaction

Lefebvre et al 1996, TAG
Thabuis et al 2003, TAG
Bonnet et al 2007, TAG



Breeding for resistance to *P. capsici* requires to transfer polygenic resistances into elite cultivars

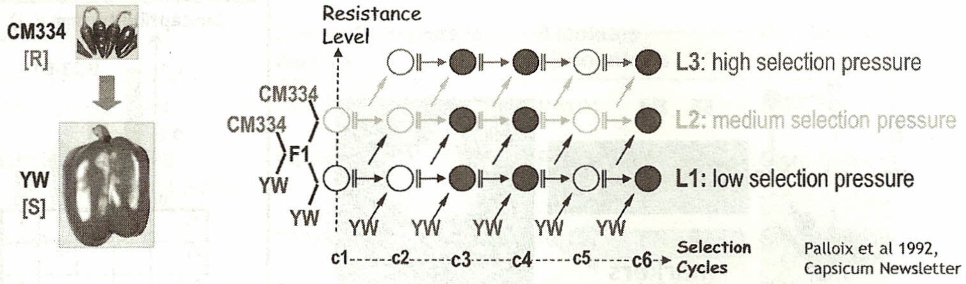
Exotic resistant germplasm
Pungent chilli pepper

Numerous cultivar types
Sweet bell pepper

A long and repeated effort !

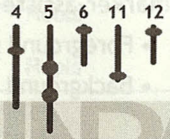
- ➢ Efficiency of the phenotypic selection
- ➢ Usefulness of the MAS

How are selected the resistance QTLs through the phenotypic selection ?



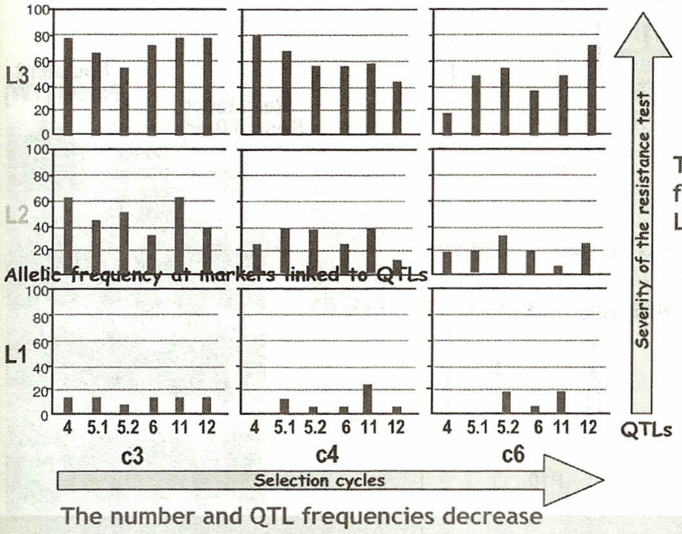
Palloix et al 1992, Capsicum Newsletter

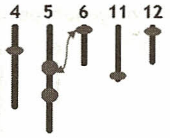
- A sort of recurrent selection scheme
  - 3 subpopulations selected with different selection pressures
  - The L1 subpop's consists of backcrossing CM334 with the recurrent YW
- An *a posteriori* analysis
  - a sample of 450 plants from 9 subpop.
  - With 50 markers for 6 QTLs and the genetic background



Thabuis et al 2004, TAG

The phenotypic selection "lost" QTLs along the selection cycles, and disrupted digenic interactions

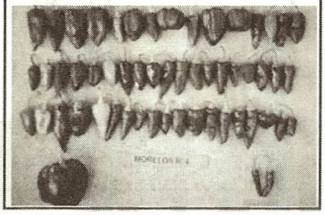




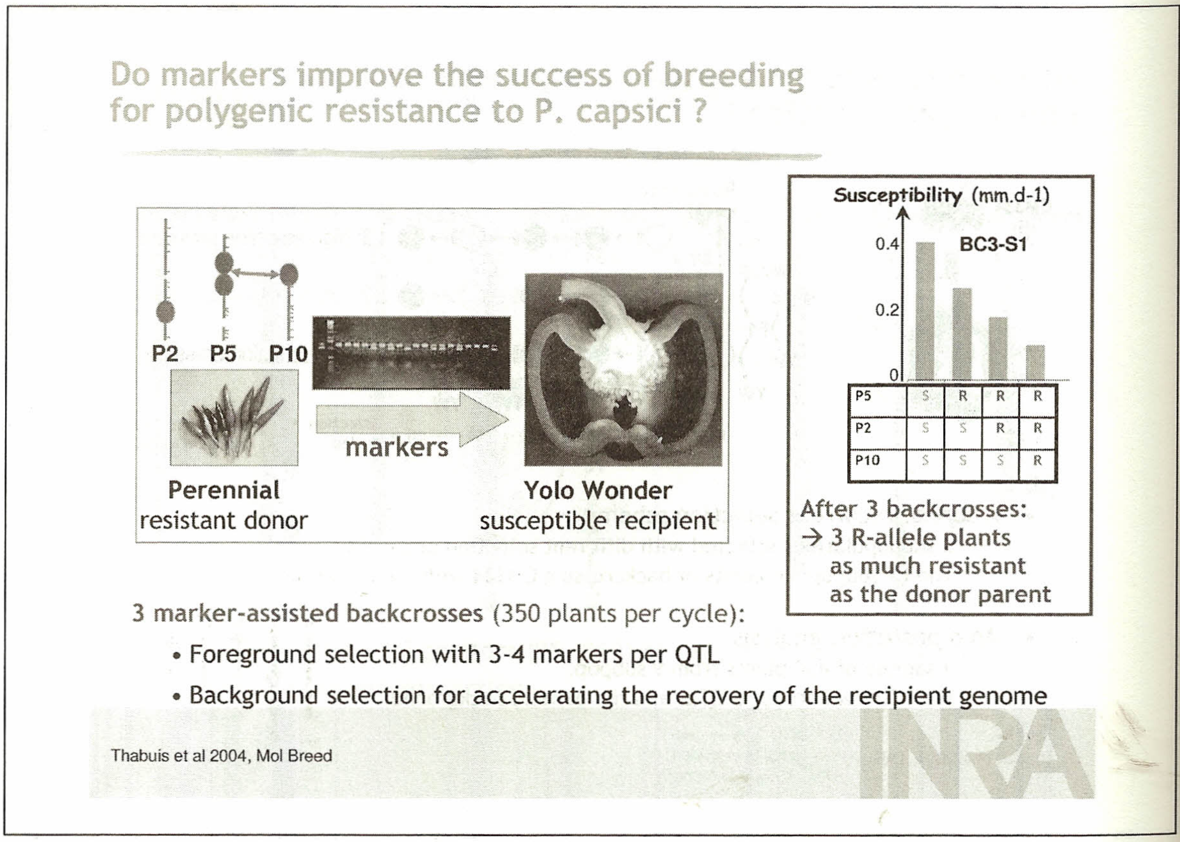
The number and QTL frequencies increase from L1 to L3

The number and QTL frequencies decrease along the selection cycles
→ QTLs and epistasis progressively lost

L3, after 6 cycles:
→ high resistance level
→ too small fruits



Thabuis et al 2004, TAG



Do markers improve the success of breeding for polygenic resistance to P. capsici ?

Perennial
resistant donor

Yolo Wonder
susceptible recipient

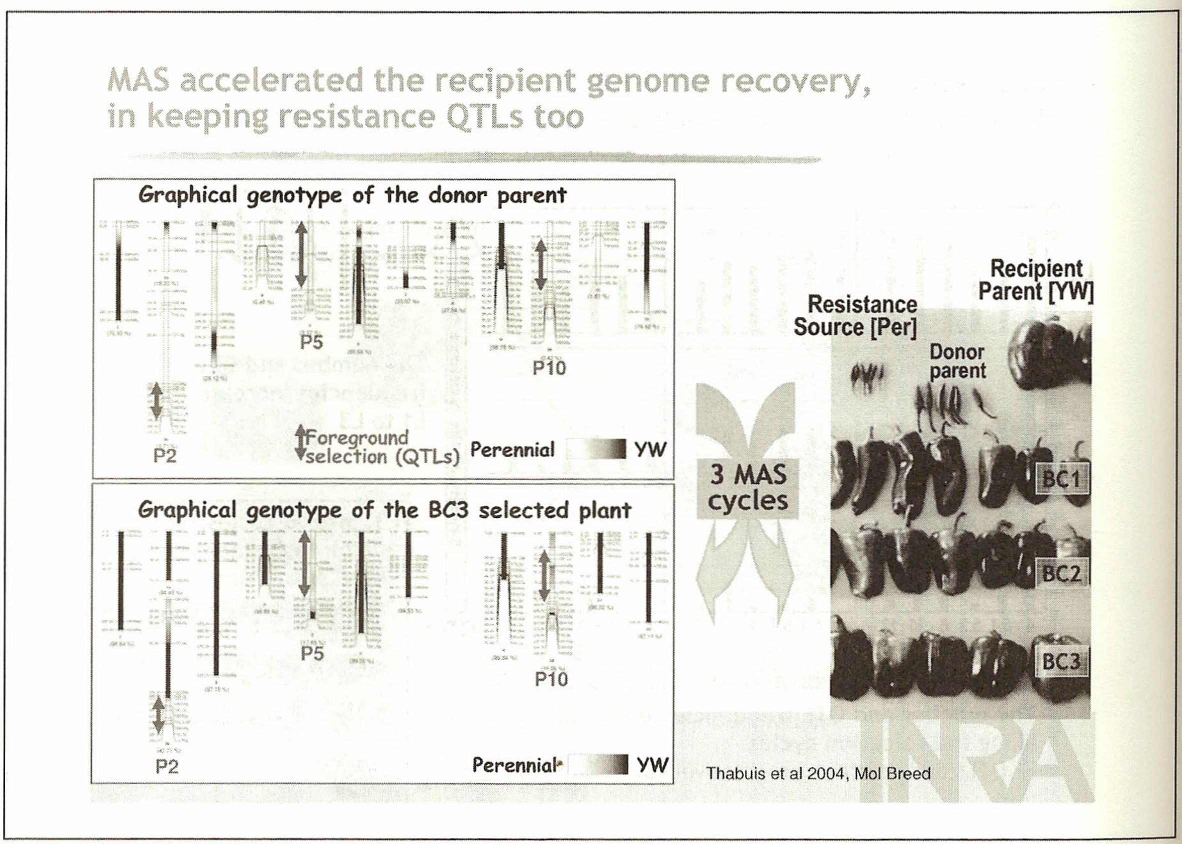
P5	S	R	R	R
P2	S	S	R	R
P10	S	S	S	R

After 3 backcrosses:
→ 3 R-allele plants as much resistant as the donor parent

3 marker-assisted backcrosses (350 plants per cycle):

- Foreground selection with 3-4 markers per QTL
- Background selection for accelerating the recovery of the recipient genome

Thabuis et al 2004, Mol Breed

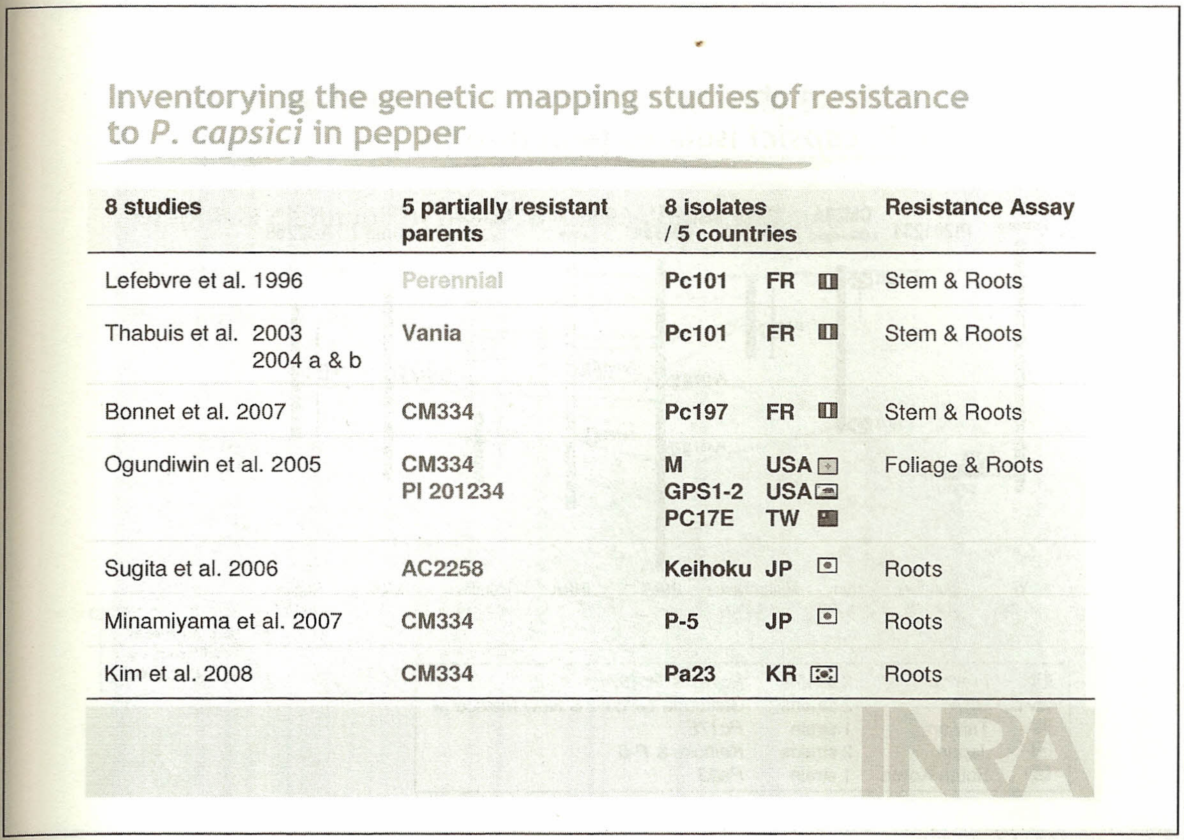


MAS accelerated the recipient genome recovery, in keeping resistance QTLs too

Graphical genotype of the donor parent

Graphical genotype of the BC3 selected plant

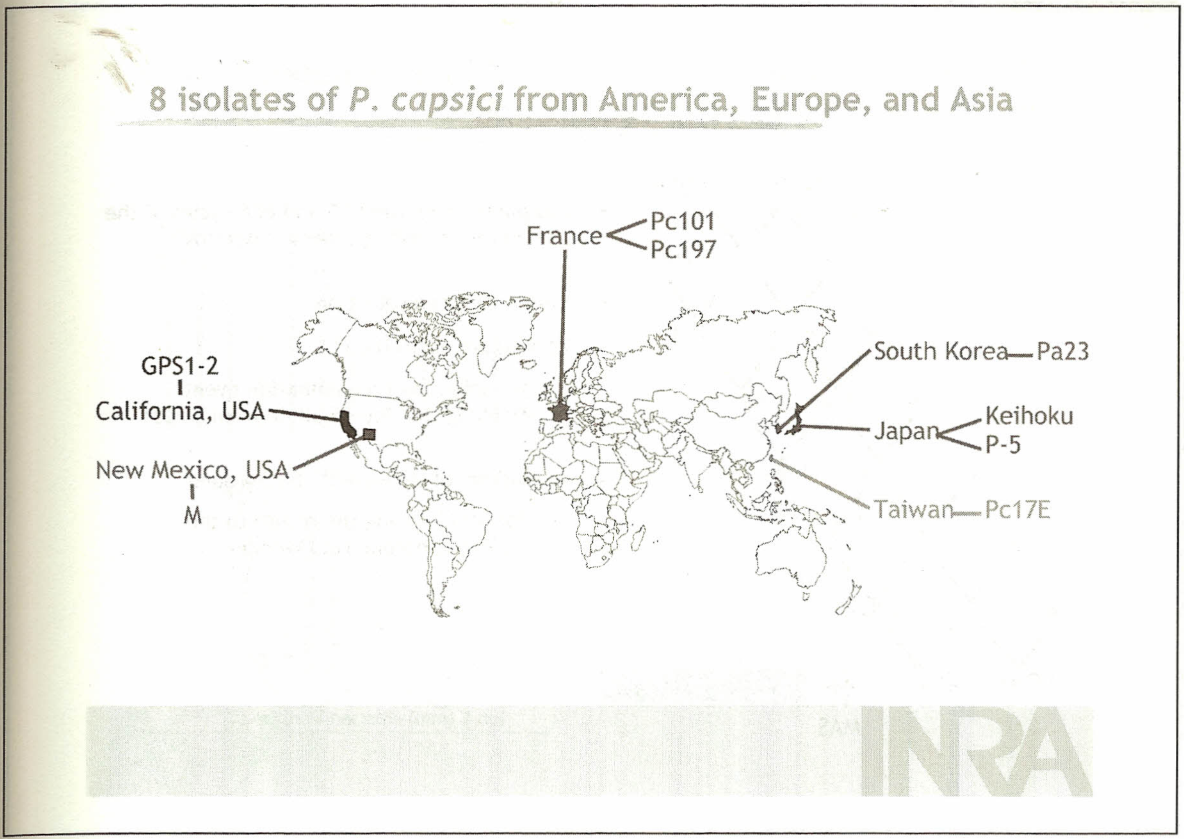
Thabuis et al 2004, Mol Breed



Inventorying the genetic mapping studies of resistance to *P. capsici* in pepper

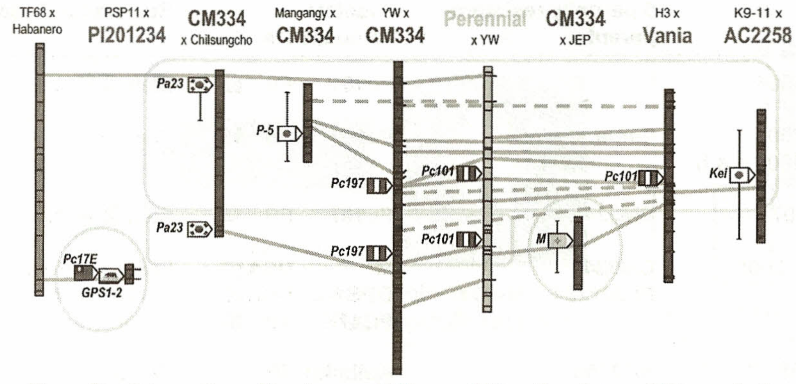
8 studies	5 partially resistant parents	8 isolates / 5 countries		Resistance Assay
Lefebvre et al. 1996	Perennial	**Pc101**	**FR**	Stem & Roots
Thabuis et al. 2003 2004 a & b	**Vania**	**Pc101**	**FR**	Stem & Roots
Bonnet et al. 2007	**CM334**	**Pc197**	**FR**	Stem & Roots
Ogundiwin et al. 2005	**CM334** **PI 201234**	**M** **GPS1-2** **PC17E**	**USA** **USA** **TW**	Foliage & Roots
Sugita et al. 2006	**AC2258**	**Keihoku**	**JP**	Roots
Minamiyama et al. 2007	**CM334**	**P-5**	**JP**	Roots
Kim et al. 2008	**CM334**	**Pa23**	**KR**	Roots

INRA



8 isolates of *P. capsici* from America, Europe, and Asia

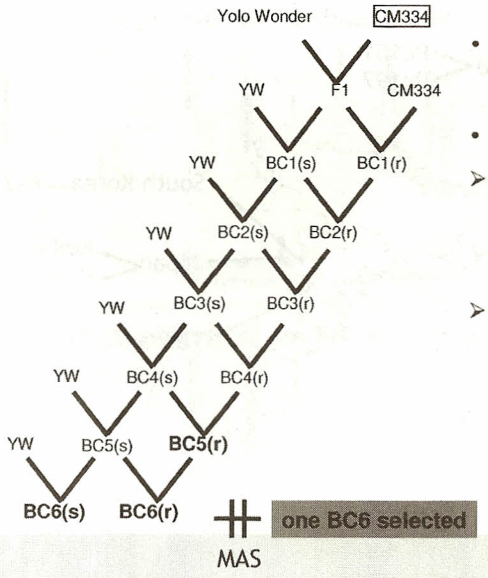
The 12 QTLs of chromosome P5 confer resistance to the 8 *P. capsici* isolates tested so far



▥	France	2 strains	*Pc101* & *Pc197*
▭ ▭	USA	2 strains	California *GPS1-2* & New Mexico *M*
■	Taiwan	1 strain	*Pc17E*
◉	Japan	2 strains	*Keihoku* & *P-5*
▣	South Korea	1 strain	*Pa23*

Map alignment facilitated by bridge markers

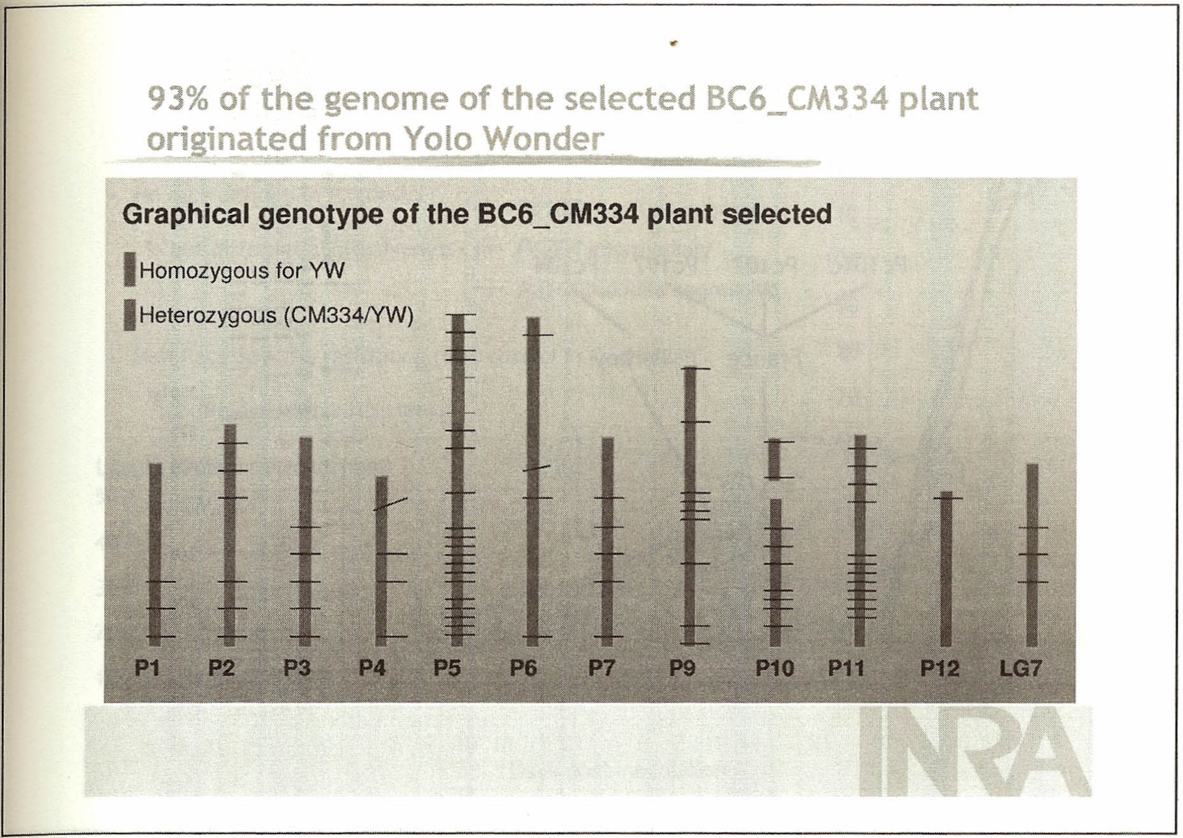
The QTL region derived from CM334 was isolated in a susceptible genetic background



- 200 plants from the BC5 and BC6 cycles of the phenotypic breeding scheme "Morelos"
- Marker-assisted selection

➢ 22 markers located on P5
  - for selecting recombination events flanking the *Phy-P5* allele from CM334

➢ 92 markers of the genetic background
  - for accelerating the return to the recipient parent Yolo Wonder

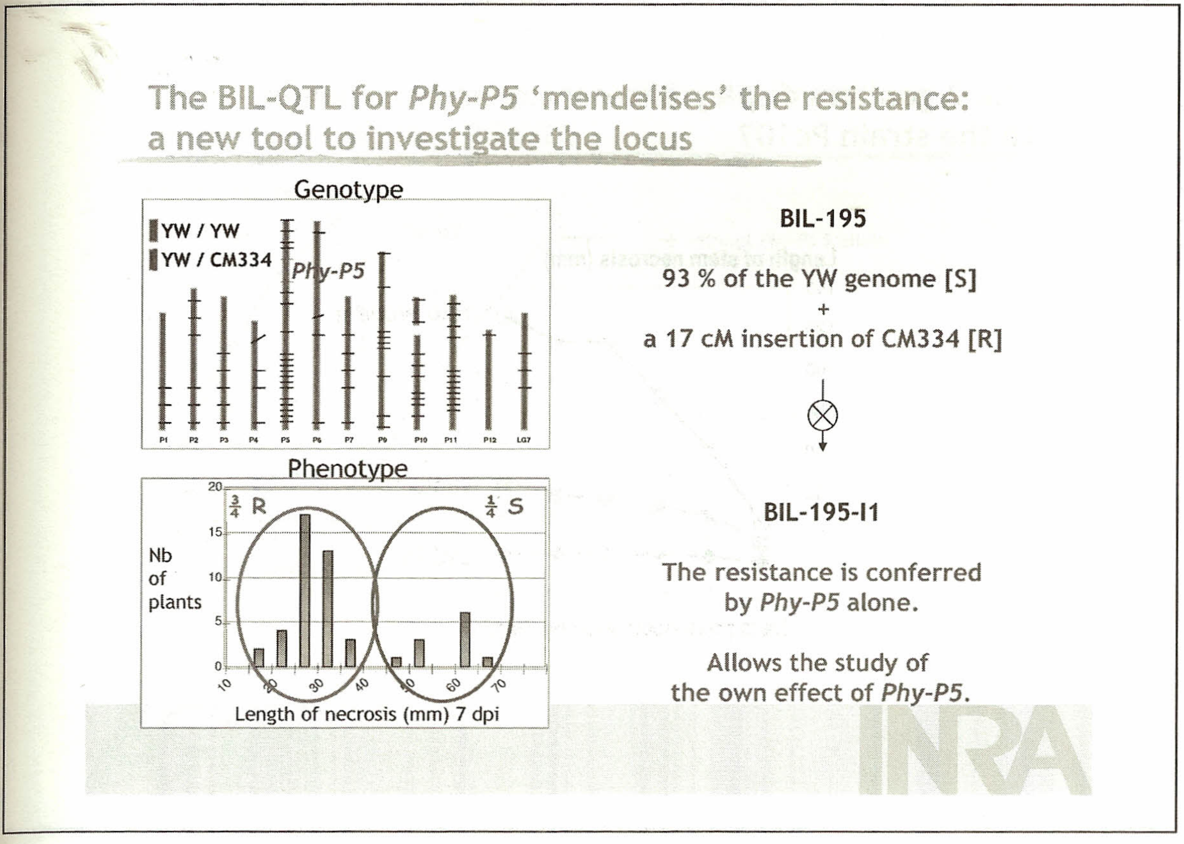


93% of the genome of the selected BC6_CM334 plant originated from Yolo Wonder

Graphical genotype of the BC6_CM334 plant selected

▮ Homozygous for YW

▮ Heterozygous (CM334/YW)



The BIL-QTL for *Phy-P5* 'mendelises' the resistance: a new tool to investigate the locus

BIL-195

93 % of the YW genome [S]

\+

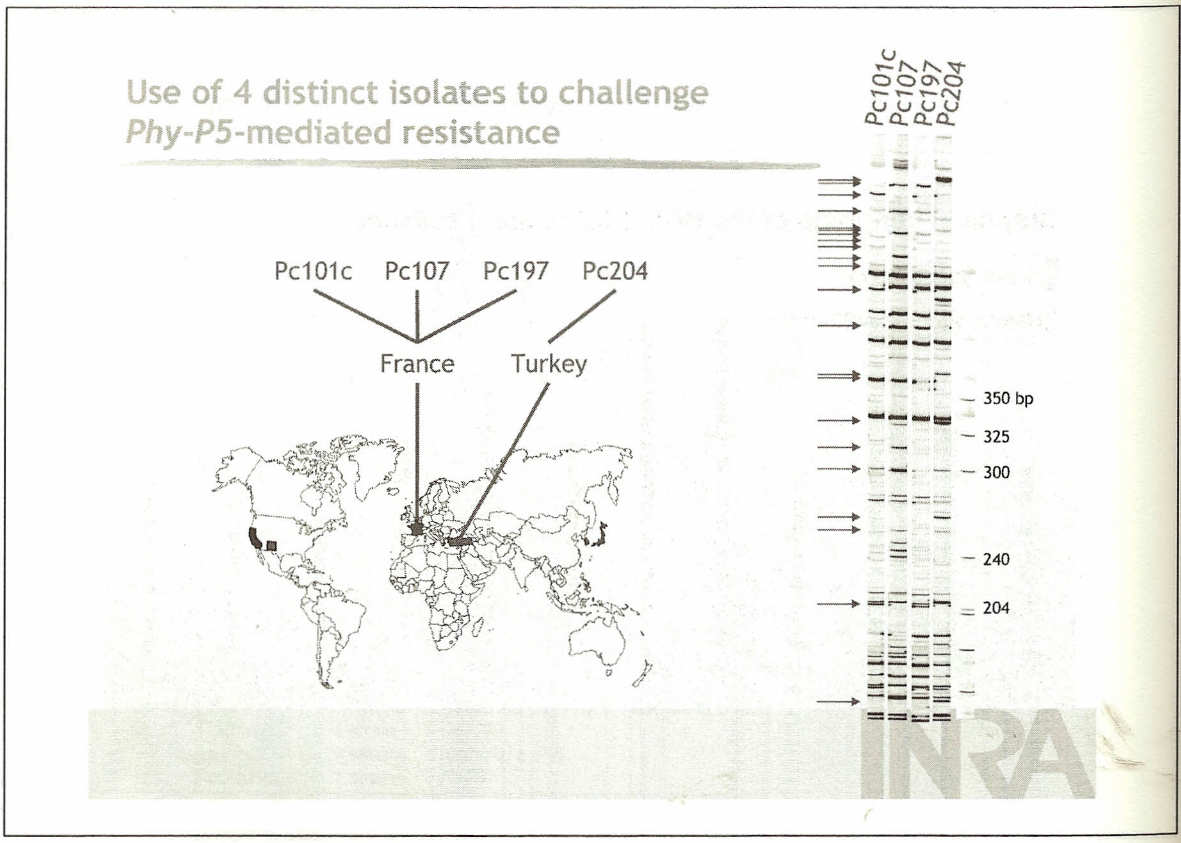
a 17 cM insertion of CM334 [R]

⊗

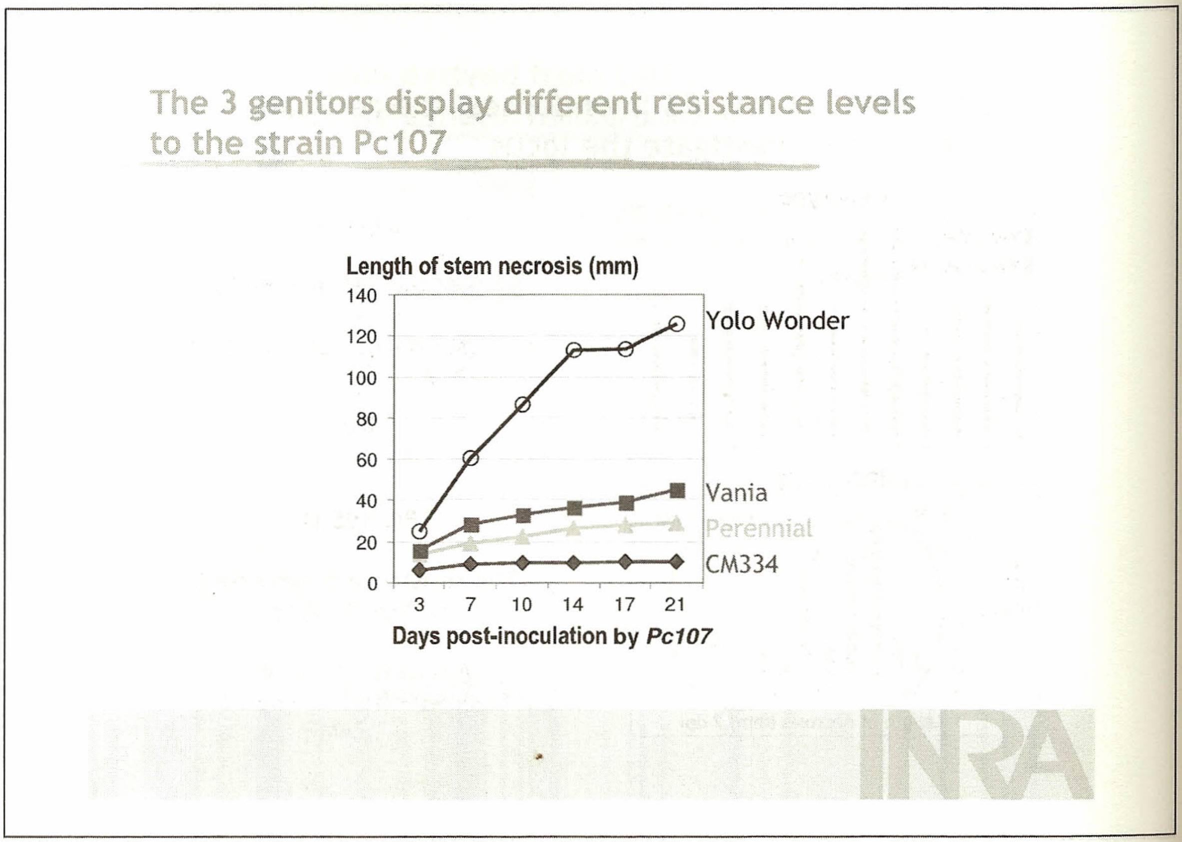
BIL-195-I1

The resistance is conferred by *Phy-P5* alone.

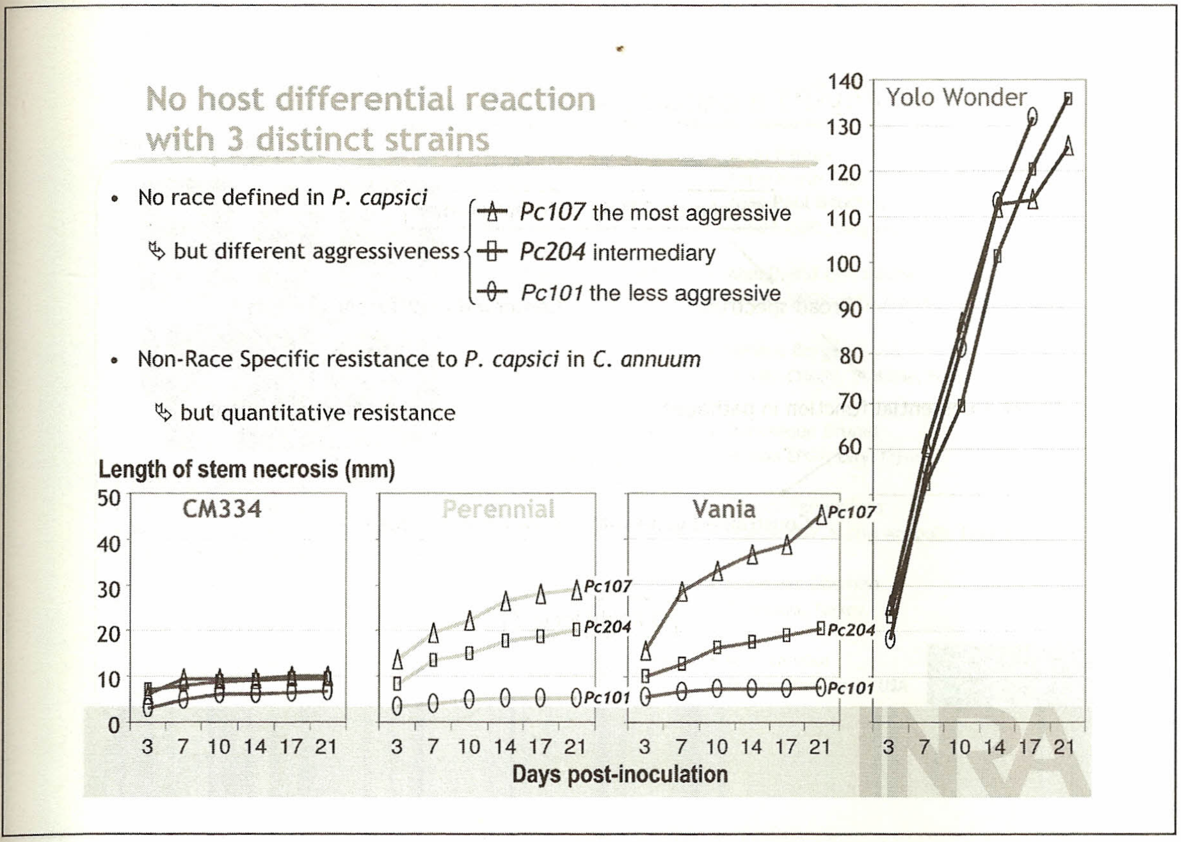
Allows the study of the own effect of *Phy-P5*.



Use of 4 distinct isolates to challenge *Phy-P5*-mediated resistance



The 3 genitors display different resistance levels to the strain Pc107



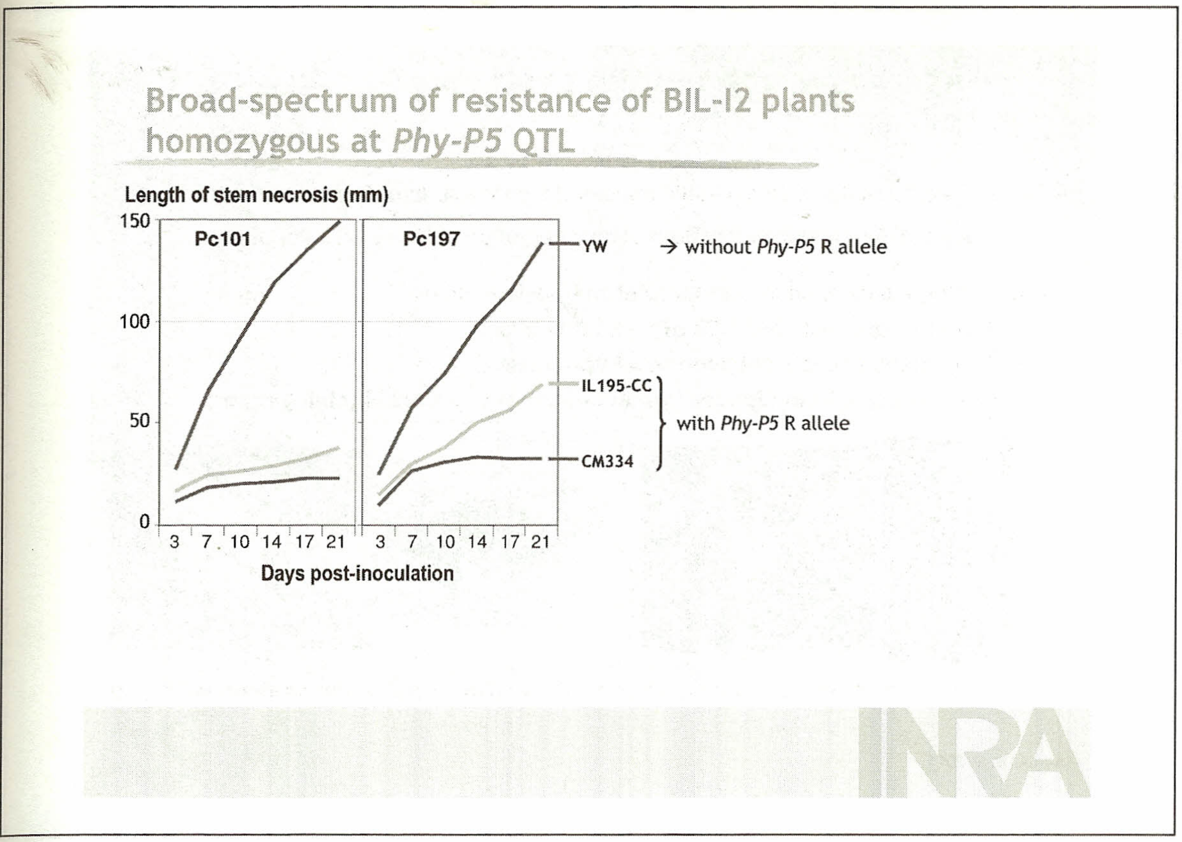
No host differential reaction with 3 distinct strains

- No race defined in *P. capsici*

  ↳ but different aggressiveness
  - *Pc107* the most aggressive
  - *Pc204* intermediary
  - *Pc101* the less aggressive

- Non-Race Specific resistance to *P. capsici* in *C. annuum*

  ↳ but quantitative resistance

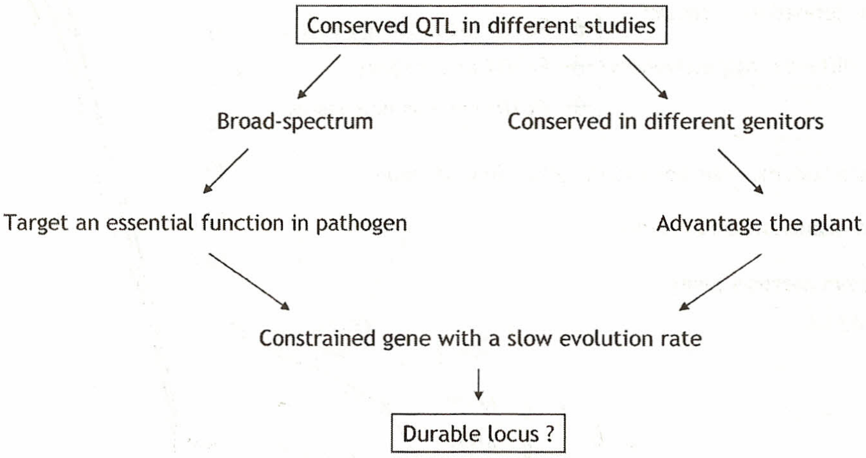


Broad-spectrum of resistance of BIL-I2 plants homozygous at *Phy-P5* QTL

YW → without *Phy-P5* R allele

IL195-CC, CM334 } with *Phy-P5* R allele

Could be *Phy-P5* a candidate QTL favoring durability ?



Conserved QTL in different studies

Broad-spectrum

Conserved in different genitors

Target an essential function in pathogen

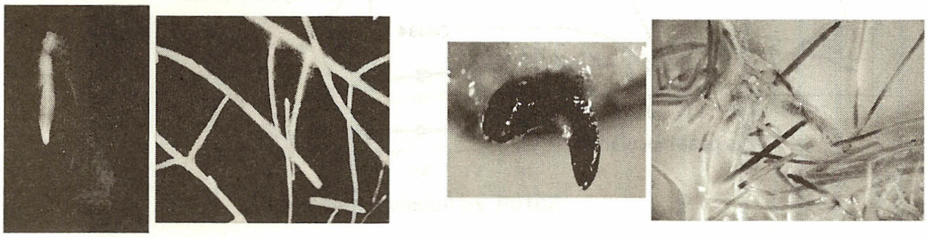
Advantage the plant

Constrained gene with a slow evolution rate

Durable locus ?

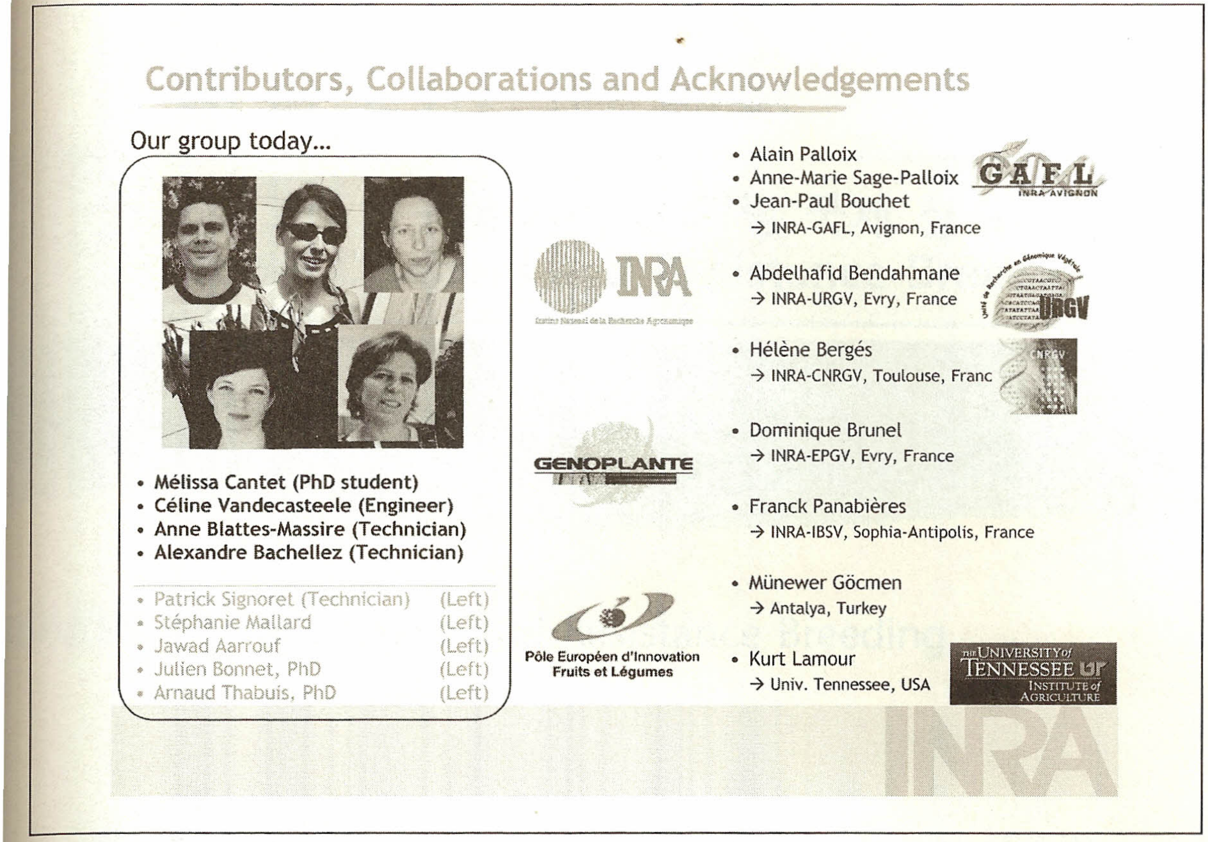
Agrobacterium rhizogenes-dependent production of co-transformed roots of pepper

- Pepper: recalcitrant to stable genetic transformation via *A. tumefaciens*
- *A. rhizogenes*-dependent co-transformation: integration of Ri + a reporter gene
- Effect of three factors on the efficiency of co-transformation :
  - Plasmidic construct : **pHKN29-gfp > pBBIN-gus**
  - Plant organ: **leaves > cotyledons > hypocotyls**
  - Plant genotype: **Yolo Wonder (sweet bell pepper) > CM334 (chili pepper)**



➢ *A. rhizogenes* transformation: an efficient alternative to study gene functions in roots

Aarrouf et al, 2011



Contributors, Collaborations and Acknowledgements

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Thank you for your attention

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