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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

Sponsors

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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*

INRA, UR 1052, Unité de Génétique et Amélioration des Fruits et Légumes (GAFL), BP 94, 84140 Montfavet, France

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.

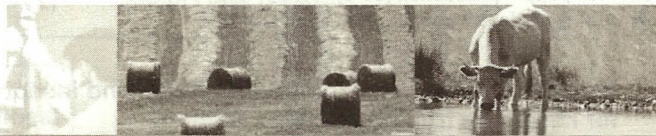
Our results, together with ongoing sequencing programs in Solanaceae and *Phytophthora*, are expected to increase the knowledge on the Solanaceae-*Phytophthora* crosstalk.

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



Véronique Lefebvre

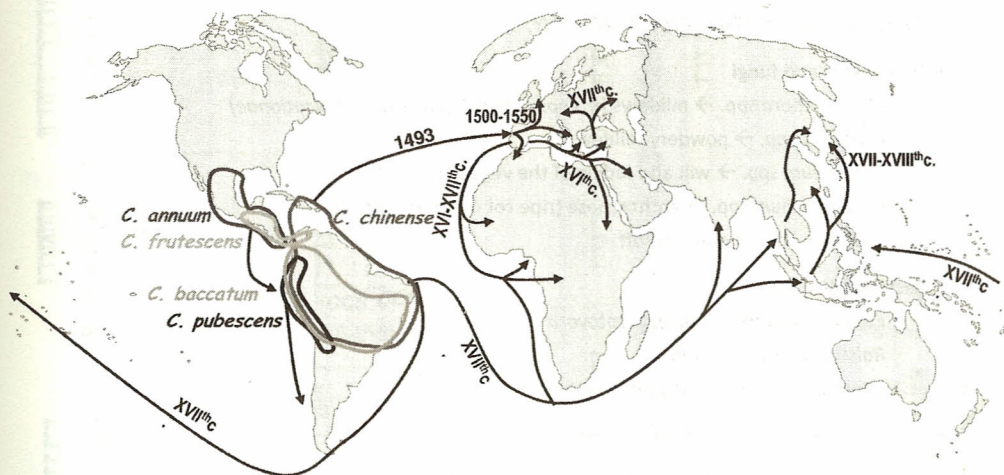


FOOD
SUSTAINABLE AGRICULTURE
ENVIRONNEMENT



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

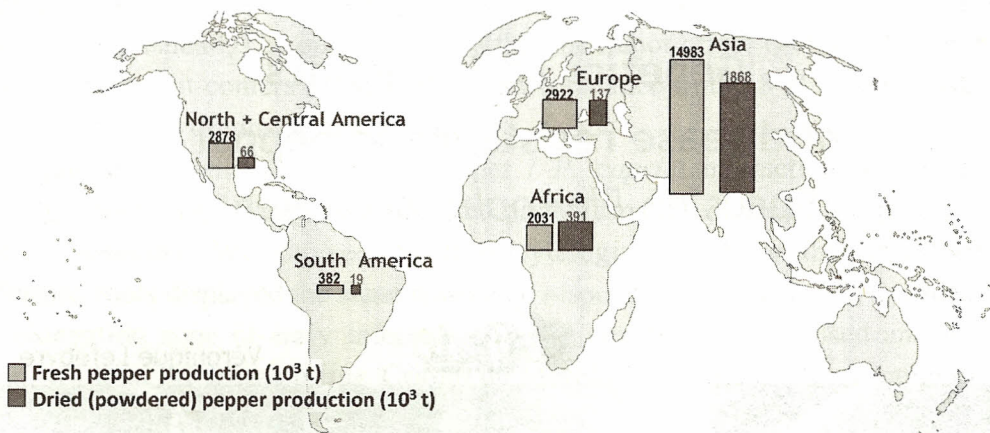
Areas of origin and main routes of spread of the 5 cultivated pepper species in the world from the XVth to XVIIIth centuries



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



Distribution of pepper production over the world cultivation areas



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

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

INRA

The geographical dispersion of the cultures exposes pepper to many parasites

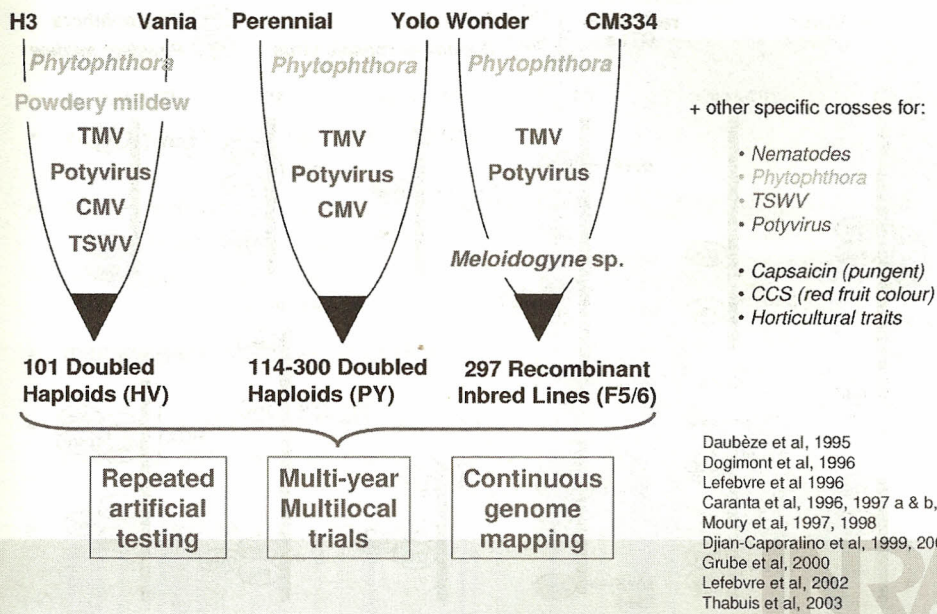
Important parasites in pepper

- 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

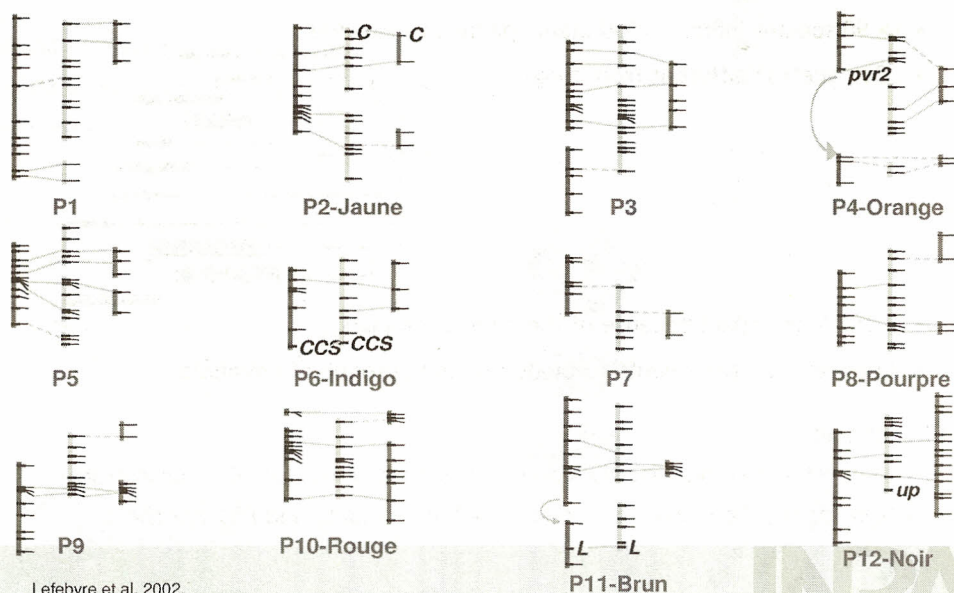
INRA

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

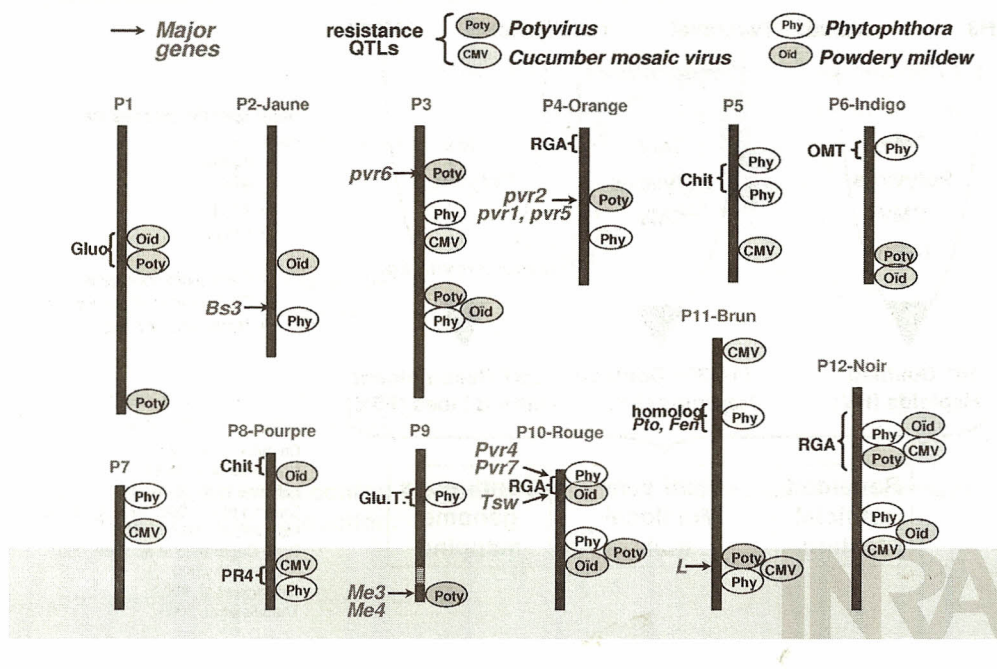


Alignment of the 3 INRA pepper intraspecific maps

→ 12 consensus linkage groups (1217 markers, including 88 anchor markers)

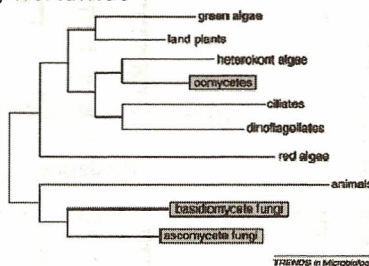


Synopsis of the distribution of the resistance genetic factors on the consensus map of pepper



Phytophthora belongs to the particular Oomycete microorganisms

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



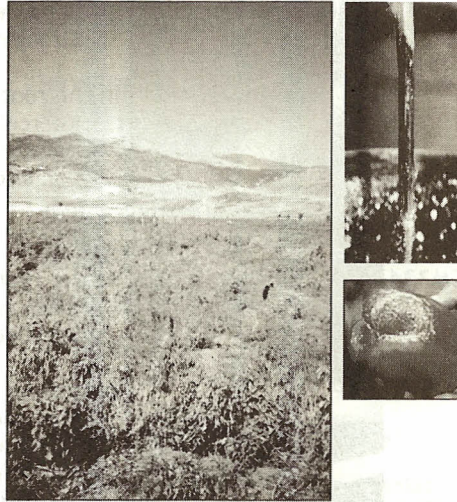
- Increasing impact because of the climate change
- Chemical soil treatments forbidden by the European Commission

➤ 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 ?



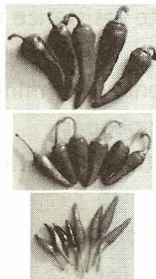
INRA

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

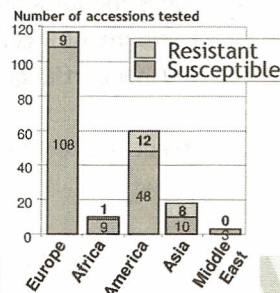
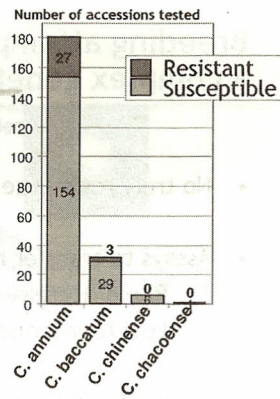
The most resistant sources:

- PI 201234 → Central America
Kimble and Grosan, 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

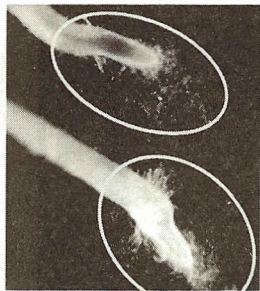
Sage-Palloix et al 2007, XIIIth Capsicum & Eggplant EUCARPIA Meeting.
Cantet and Lefebvre, unpublished.



INRA

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

YW
 [S]



At the root apex:

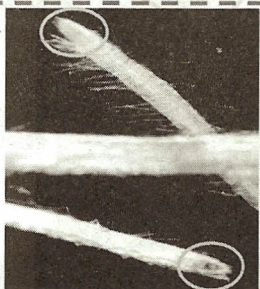
- Dense mycelial development
- Strong necrosis

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
 → reinforce the tissue



Photos: J. Aarouf

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

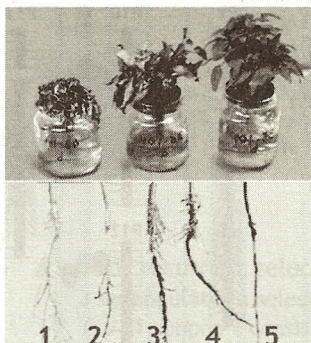
➤ dissect the complex resistance into elementary components

↓
 perform QTL analyses

2 resistance tests supply 4 elementary components

Root inoculation

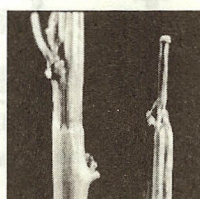
Extent of root necrosis (1 to 5)
1 semi-quantitative criterion



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 $h^2_{BS} = 0,93$

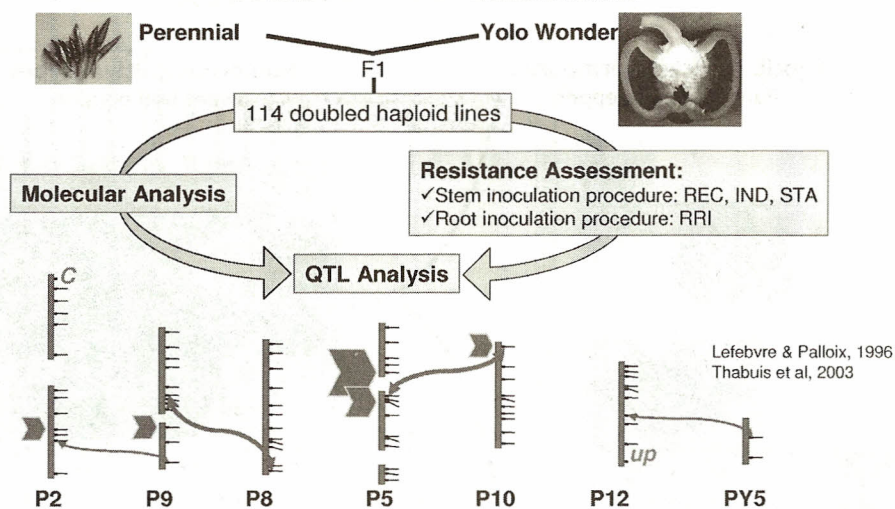
h^2_{BS} for *P. capsici* Pc197 resistance in F5YC RIL progeny

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

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



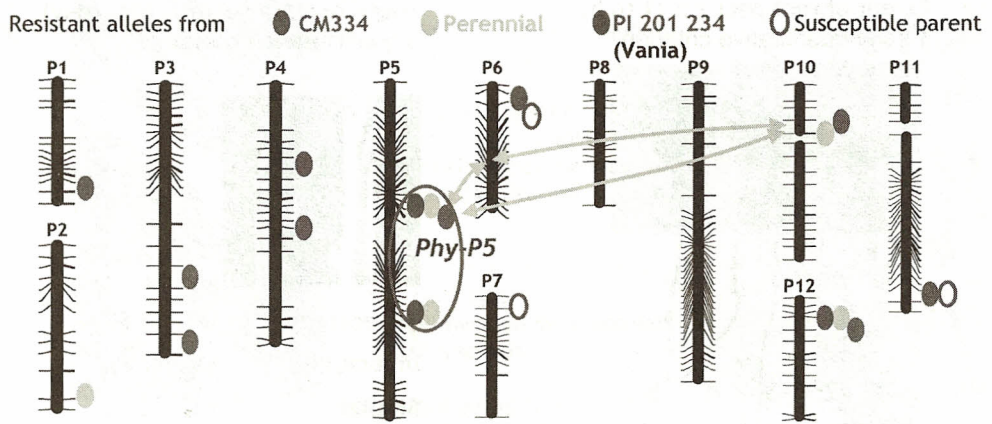
Both additive and epistatic QTLs control the resistance to *P. capsici* in pepper



➤ 5 additive QTLs and 4 epistatic relationships



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



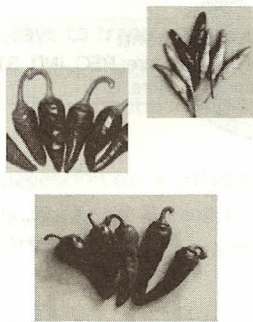
- QTLs common to 2-3 genitors (P5, P12) or specific-genitor QTLs
- Significant digenic interaction

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

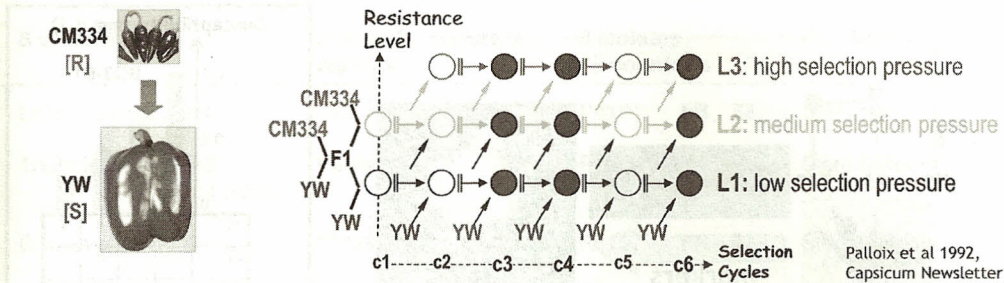


A long and repeated effort !

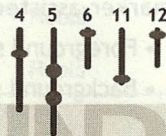
- Efficiency of the phenotypic selection
- Usefulness of the MAS



How are selected the resistance QTLs through the phenotypic selection ?



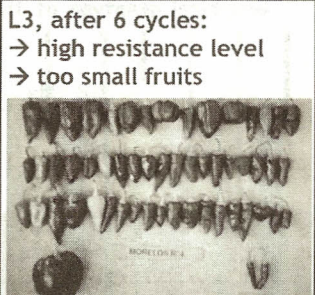
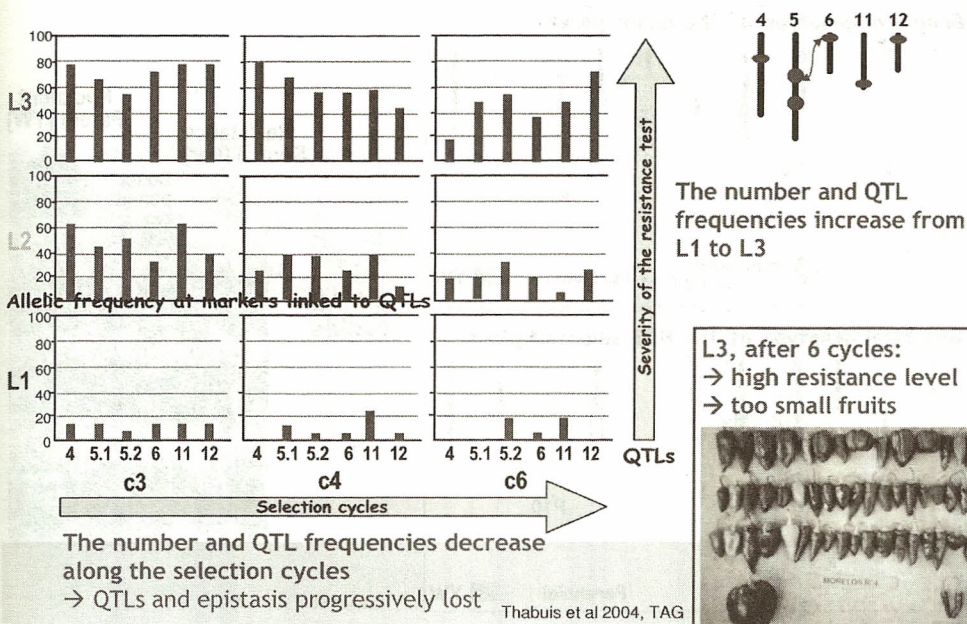
- A sort of recurrent selection scheme
 - 3 subpopulations selected with different selection pressures
 - The L1 subpop's consists of backcrossing CM334 with the recurrent YW
- An *a posteriori* analysis
 - a sample of 450 plants from 9 subpop.
 - With 50 markers for 6 QTLs and the genetic background



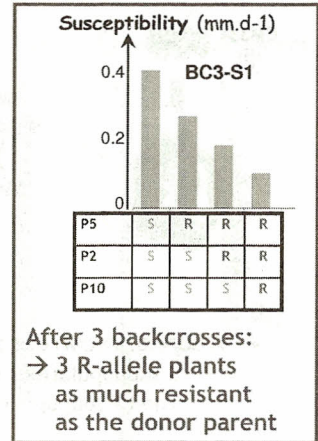
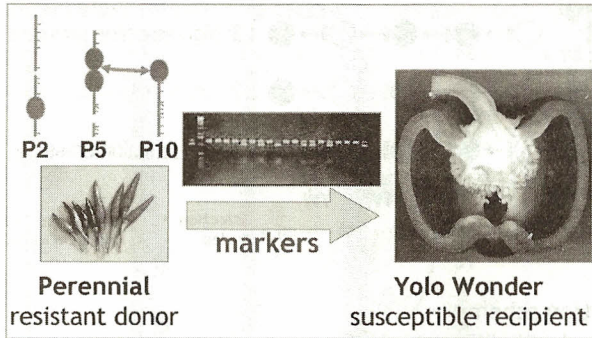
Thabuis et al 2004, TAG



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



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

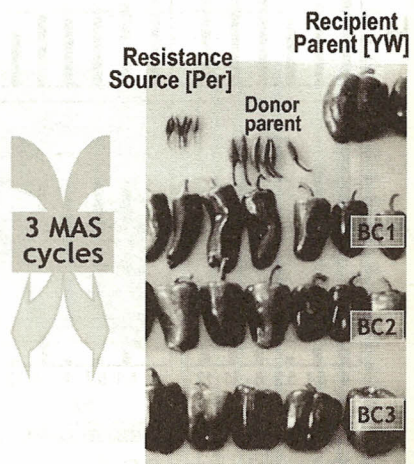
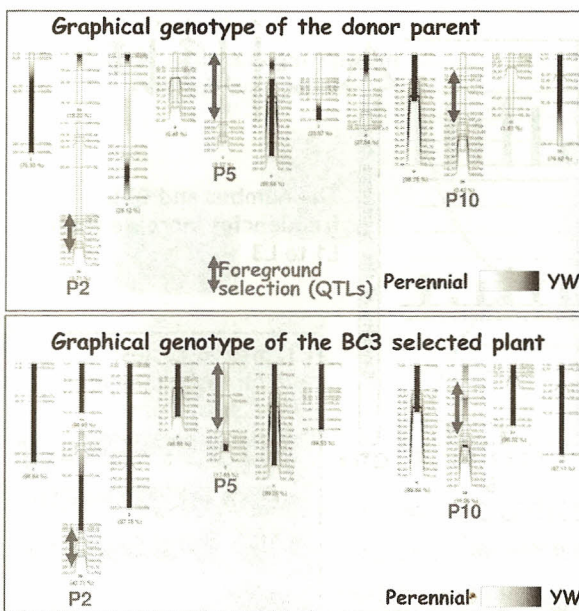


3 marker-assisted backcrosses (350 plants per cycle):

- 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



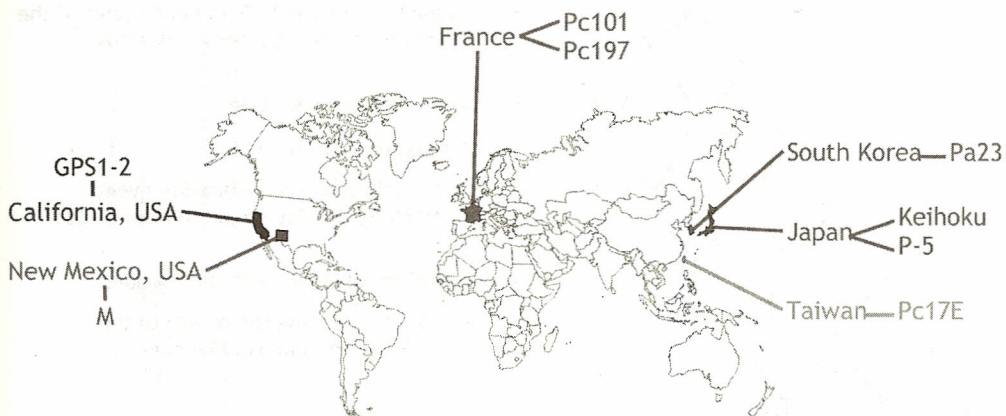
Thabuis et al 2004, Mol Breed

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

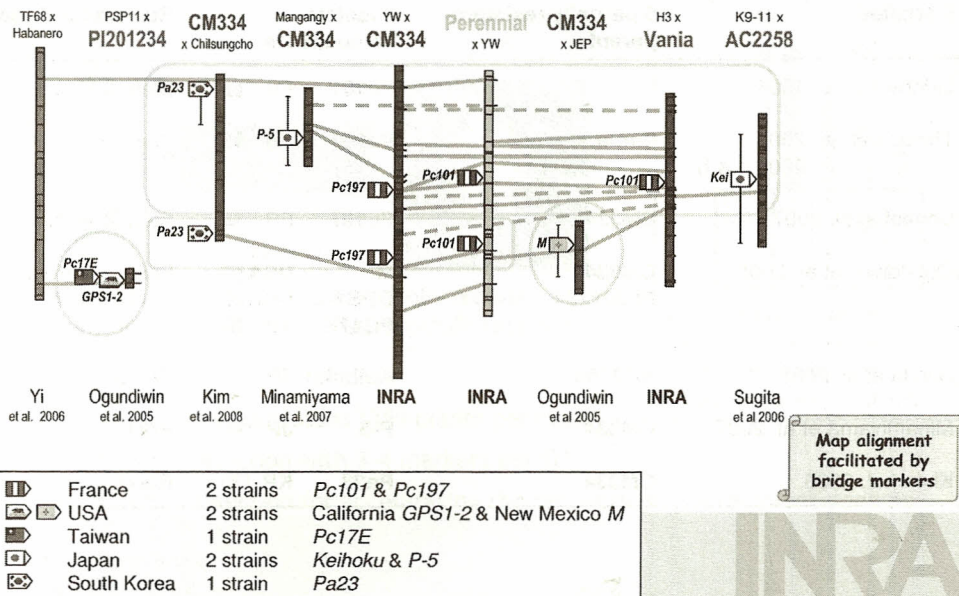
8 studies	5 partially resistant parents	8 isolates / 5 countries	Resistance Assay
Lefebvre et al. 1996	Perennial	Pc101 FR ■	Stem & Roots
Thabuis et al. 2003 2004 a & b	Vania	Pc101 FR ■	Stem & Roots
Bonnet et al. 2007	CM334	Pc197 FR ■	Stem & Roots
Ogundiwin et al. 2005	CM334 PI 201234	M USA □ GPS1-2 USA □ PC17E 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



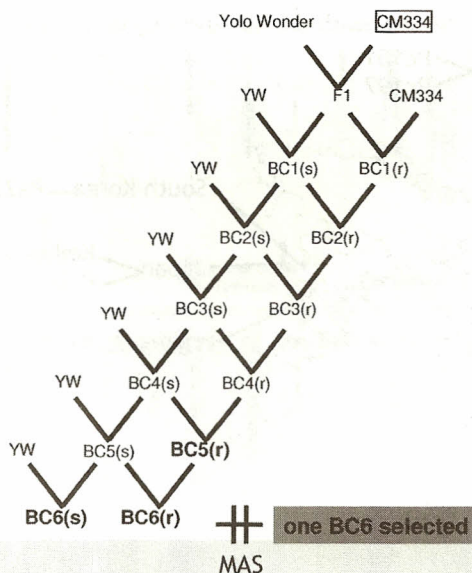
8 isolates of *P. capsici* from America, Europe, and Asia



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



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

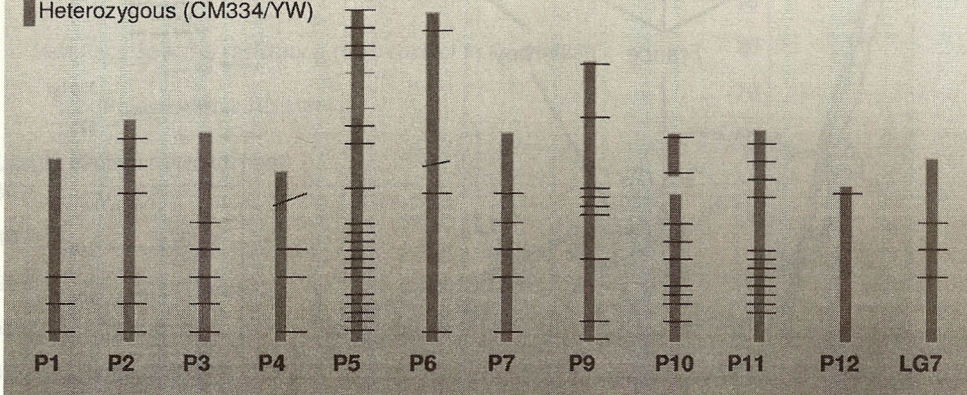


- 200 plants from the BC5 and BC6 cycles of the phenotypic breeding scheme "Morelos"
- Marker-assisted selection
- 22 markers located on P5
 - for selecting recombination events flanking the *Phy-P5* allele from CM334
- 92 markers of the genetic background
 - for accelerating the return to the recipient parent Yolo Wonder

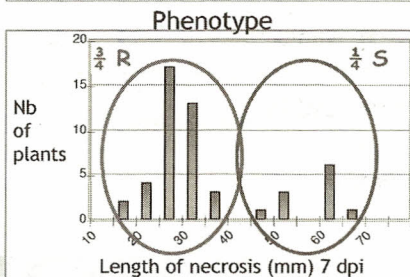
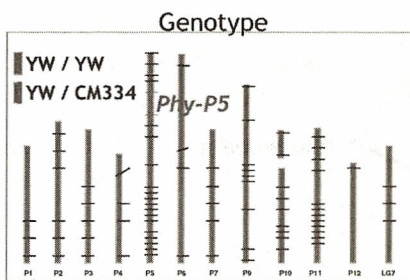
93% of the genome of the selected BC6_CM334 plant originated from Yolo Wonder

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]



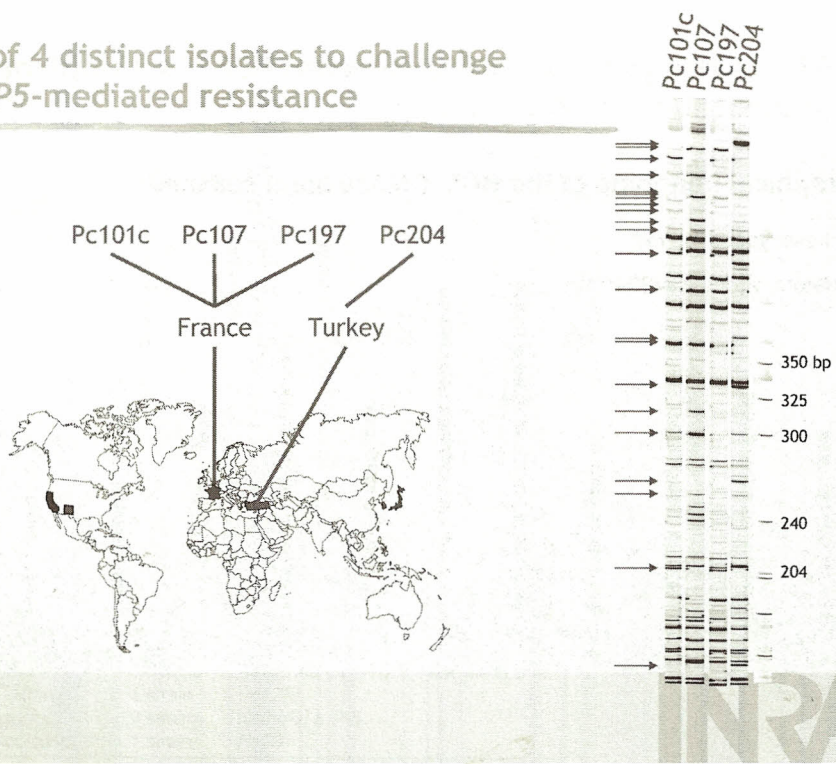
BIL-195-I1

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

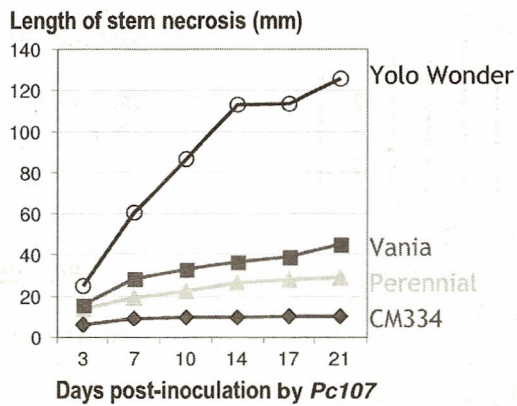
Allows the study of the own effect of *Phy-P5*.



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



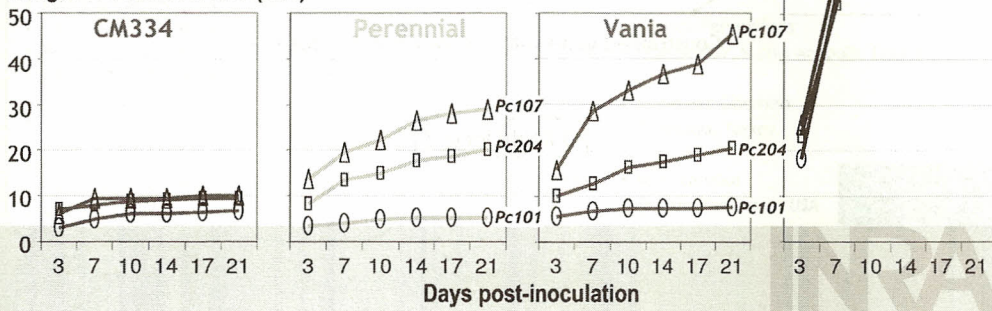
The 3 genitors display different resistance levels
 to the strain Pc107



No host differential reaction with 3 distinct strains

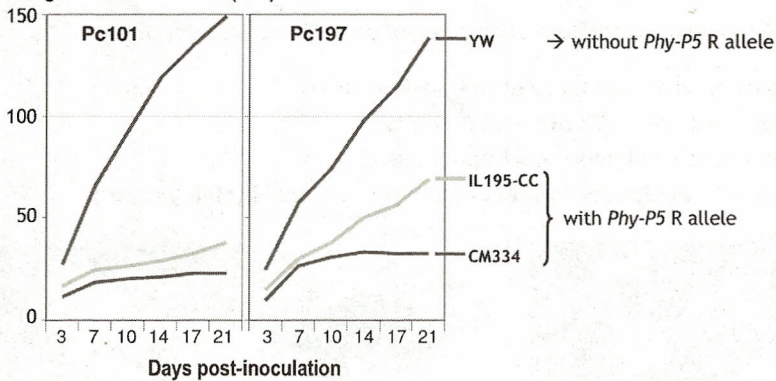
- No race defined in *P. capsici*
 - ↳ but different aggressiveness
 - △ *Pc107* the most aggressive
 - *Pc204* intermediary
 - *Pc101* the less aggressive
- Non-Race Specific resistance to *P. capsici* in *C. annuum*
 - ↳ but quantitative resistance

Length of stem necrosis (mm)

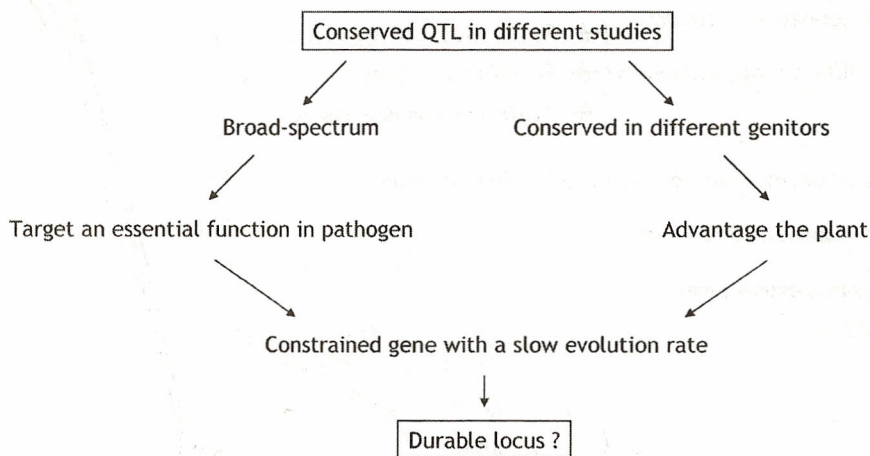


Broad-spectrum of resistance of BIL-12 plants homozygous at *Phy-P5* QTL

Length of stem necrosis (mm)

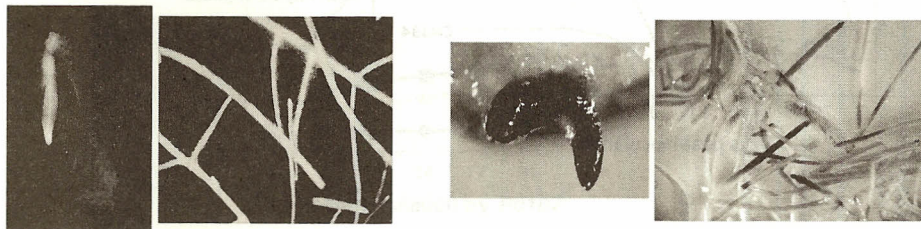


Could be *Phy-P5* a candidate QTL favoring durability ?



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)**

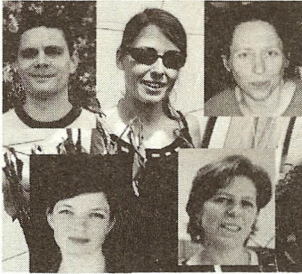


➤ *A. rhizogenes* transformation: an efficient alternative to study gene functions in roots

Aarrouf et al, 2011

Contributors, Collaborations and Acknowledgements

Our group today...



- Mélissa Cantet (PhD student)
- Céline Vandecasteele (Engineer)
- Anne Blattes-Massire (Technician)
- Alexandre Bachellez (Technician)

- Patrick Signoret (Technician) (Left)
- Stéphanie Mallard (Left)
- Jawad Aarouf (Left)
- Julien Bonnet, PhD (Left)
- Arnaud Thabuis, PhD (Left)



- Alain Palloix
- Anne-Marie Sage-Palloix
- Jean-Paul Bouchet
→ INRA-GAFL, Avignon, France



- Abdelhafid Bendahmane
→ INRA-URGV, Evry, France



- Hélène Bergés
→ INRA-CNRGV, Toulouse, France

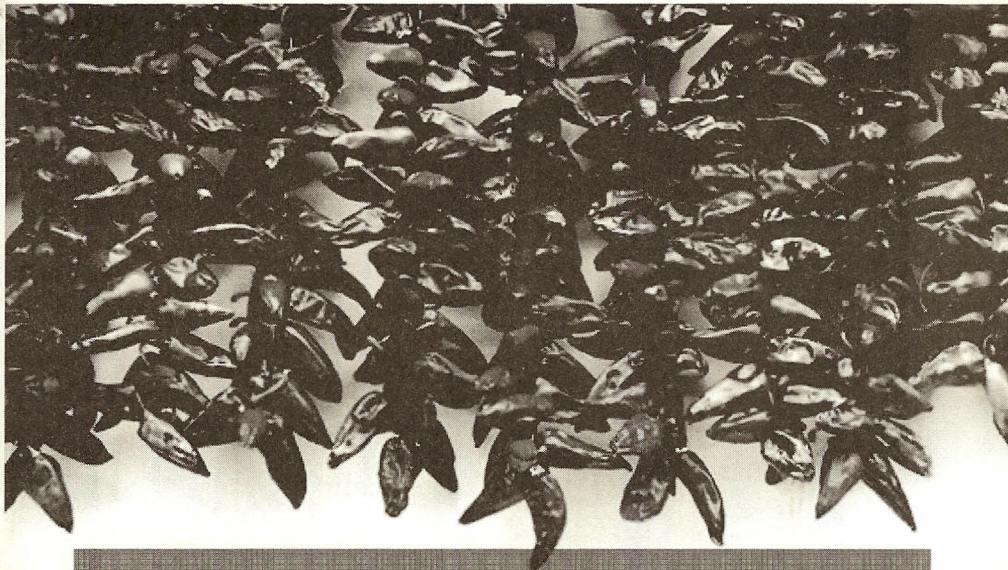


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- Münewer Göcmen
→ Antalya, Turkey

- Kurt Lamour
→ Univ. Tennessee, USA



Thank you for your attention

