Genetics and genomics of disease resistance in pepper: focus on Phytophthora threats
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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
Dongbu Hannong, Nongwoo Bio, Jeil Seed,
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Fungi and Plants, Samrang A.T.I., Sakata Korea,
Takii Korea, Hyundai Seed, Samsung Seed

Website | www.capsicum.or.kr
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

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

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

H3 Vania Perennial Yolo Wonder CM334
Powdery mildew

TMV
Potyvirus
CMV
TSVV

Phytophthora

(TMV Potyvirus CMV)

Meloidogyne sp.

+ other specific crosses for:
- Nematodes
- Phytophthora
- TSWV
- Potyvirus
- Capsaicin (pungent)
- CCS (red fruit colour)
- Horticultural traits

101 Doubled Haploids (HV) 114-300 Doubled Haploids (PY) 297 Recombinant Inbred Lines (F5/6)

Repeated artificial testing
Multi-year Multilocal trials
Continuous genome mapping

Alignment of the 3 INRA pepper intraspecific maps

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

P1

P5

P9

P2-Jaune

P6-Indigo

P10-Rouge

P3

P7

P11-Brun

P4-Orange

P8-Pourpre

P12-Noir

Lefebvre et al., 2002. Barchi et al., 2008

Daubliev et al., 1995
Dorgan et al., 1996
Lefebvre et al., 1998
Carrata et al., 1996, 1997 a & b, 2002
Moury et al., 1997, 1998
Djian-Caporalino et al., 1999, 2001
Guibe et al., 2000
Lefebvre et al., 2002
Thabuis et al., 2003
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
  Kibble 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

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

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

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

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

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

Photos: J. Aarruf

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2 resistance tests supply 4 elementary components

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

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

Receptivity $h^2_{BS} = 0.87$
Inducibility $h^2_{BS} = 0.88$
Stability $h^2_{BS} = 0.93$

Mean root rot index $h^2_{BS} = 0.69$

> continuous distribution, transgressive individuals and high $h^2$ values in 5 x R progenies

Lefebvre and Pallaix 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 QTL Analysis

Resistance Assessment:
- Stem inoculation procedure: REC, IND, STA
- Root inoculation procedure: RRI

Lefebvre & Pallaix, 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

<table>
<thead>
<tr>
<th>Resistant alleles from</th>
<th>CM334</th>
<th>Perennial</th>
<th>PI 201 234</th>
<th>Susceptible parent</th>
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</thead>
<tbody>
<tr>
<td>P1</td>
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<td>P11</td>
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</tbody>
</table>

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

Lelebvre 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

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

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

- The number and QTL frequencies increase from L1 to L3
  - high resistance level
  - too small fruits
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

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

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

Thabuis et al 2004, Mol Breed
### Inventoried Genetic Mapping Studies of Resistance to *P. capsici* in Pepper

<table>
<thead>
<tr>
<th>8 studies</th>
<th>5 partially resistant parents</th>
<th>8 isolates / 5 countries</th>
<th>Resistance Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lefebvre et al. 1996</td>
<td>Perennial</td>
<td>Pc101 FR</td>
<td>Stem &amp; Roots</td>
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<tr>
<td>Thabuis et al. 2003</td>
<td>Vania</td>
<td>Pc101 FR</td>
<td>Stem &amp; Roots</td>
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<td>2004 a &amp; b</td>
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<tr>
<td>Bonnet et al. 2007</td>
<td>CM334</td>
<td>Pc197 FR</td>
<td>Stem &amp; Roots</td>
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<tr>
<td>Ogundiwon et al. 2005</td>
<td>CM344</td>
<td>M USA GPS1-2 USA PC17E TW</td>
<td>Foliage &amp; Roots</td>
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<td>Pi 201234</td>
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<td>Sugita et al. 2006</td>
<td>AC2258</td>
<td>Keihoku JP</td>
<td>Roots</td>
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<td>Minamiyama et al. 2007</td>
<td>CM334</td>
<td>P-5 JP</td>
<td>Roots</td>
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<tr>
<td>Kim et al. 2008</td>
<td>CM334</td>
<td>Pa23 KR</td>
<td>Roots</td>
</tr>
</tbody>
</table>

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

- **France** - Pc101, Pc197
- **South Korea** - Pa23
- **Japan** - Keihoku, P-5
- **Taiwan** - Pc17E
- **California, USA** - GPS1-2
- **New Mexico, USA** - M

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- 217 -
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

Genotype

- YW / YW
- YW / CM334
- Phy-P5

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*  
  - *Pc107* the most aggressive  
  - *Pc204* intermediary  
  - *Pc101* the less aggressive

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

Length of stem necrosis (mm)

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

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**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

Aarrof et al, 2011
Contributors, Collaborations and Acknowledgements

Our group today...

- Alain Palloix
- Anne-Marie Sage-Palloix
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- Arnaud Thabuis, PhD  (Left)

Thank you for your attention