Brassica Genetics & Genomics

Research Program at INRA

Michel RENARD

INRA Research Program, Saskatoon, December 10th, 2009

Brassica Group

- Anne-Marie Chèvre
- Anne Laperche
- M. Renard
- Régine Delourme
- Maria Manzanarès
- Antoine Gravot
- Alain Bouchereau
- Nathalie Nesi
- Mathilde Orsel
- Françoise Le Cahérec
- …..

40 persons from
- INRA
- Agrocampus Ouest
- University of Rennes

Actors & Partnership

- Arabidopsis group in Versailles
- Other INRA Divisions
- Universities, CNRS, ….
- Biogenouest core facilities

- Promosol, Sofiprotéol
- Cetiom, Onidol
- GIE Colza & Procolza
- Génoplante & Biogemma
- Geves
- Ademe, …

Genetic Resources

Anne Laperche & M. Renard

National Center for Brassica Genetic Resources








National Collection
B. oleracea : 563 accessions
B. napus 58 accessions

Network Collection
B. napus : 18 accessions

INRA Collection
B. oleracea : 479 accessions
B. napus : 726 accessions
B. carinata : 57 accessions
B. nigra : 3 accessions
B. juncea : 130 accessions

European Collections
RESGEN Project
Vavilov

Evaluation and multiplication

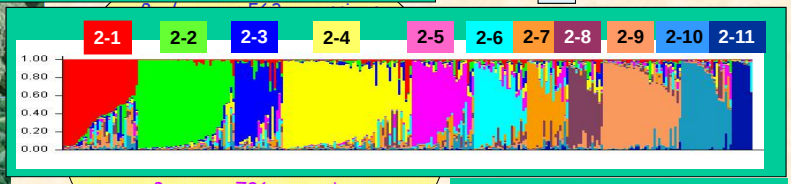
Structuration of genetic diversity

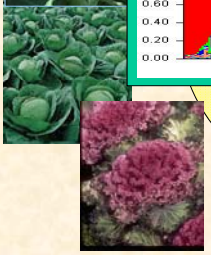
B. oleracea : 396 accessions
B. napus : 380 accessions
B. rapa : 96 accessions

In Progress…

National Center for Genetic Resources

Core collections





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European Collections
RESGEN Project
Vavilov

Structuration of genetic diversity

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EMS population for TILLING

Nathalie Nesi

'Tanto'

1- Germination after EMS treatment

2- M1 plants before vernalisation

3- M1 plants in a plastic tunnel

6- M2 collection

5- Production of M2 seeds

4- Notations of M1 plants

In Progress…

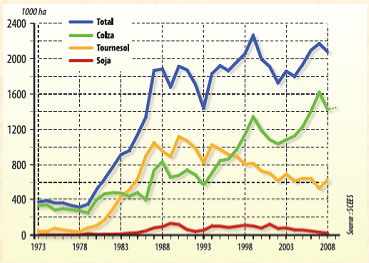
➢**Development of genetic materials**

e.g. Populations for Nested Association Mapping

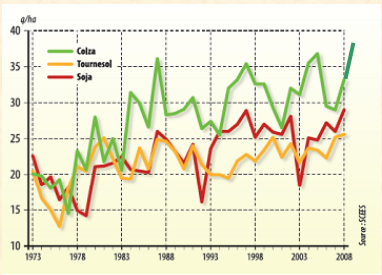
Oilseed rape : an increasing economic importance

WOSR production in France:

Evolution of acreage



Evolution of yield



Main traits of interest

- **Oil Yield**
- **Abiotic stress resistance: nitrogen, water**
- **Disease Resistance: blackleg, clubroot, broomrape**

Three Brassica teams

- Genome organisation
- Oil yield under nitrogen and water limitations
- Disease resistance

Genome Organisation
team

Anne-Marie Chèvre

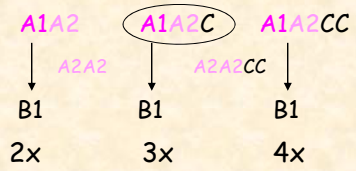
Genome Organisation

1)Homologous & Homoeologous Recombination

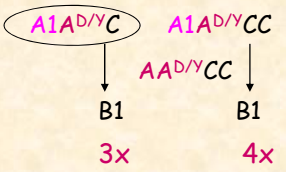
Homologous recombination: analysis of A genome

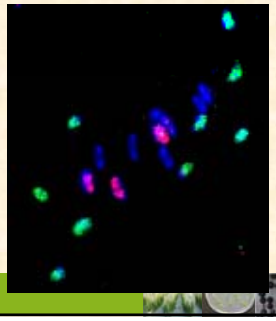
Analysis of the A genome : Production of F1 hybrids at different ploidy levels (2x/3x/4x) between the same genotypes and analyses of the homologous recombination rates.

Effect of the ploidy level (*B.rapa*)

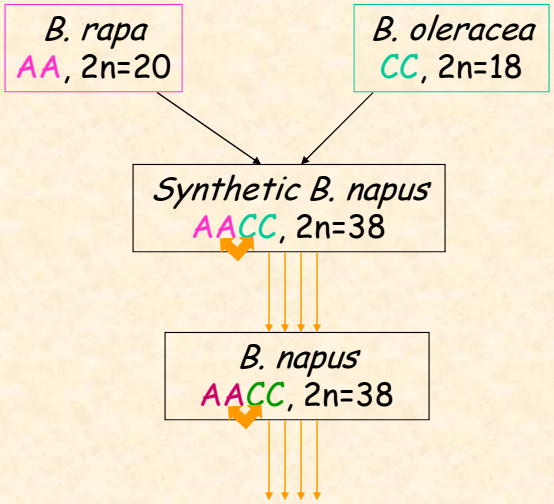


Effect of the genotype (*B.rapa*/ *B.napus*)





Homoeologous recombination



Results & Perspectives

Homologous recombination between A genome : AA<AACC<<AAC

-> introgression can be restricted to the genes of interest

- Impact of number and nature of the C chromosomes?
- What about CCA hybrids?

Homoeologous recombination between A and C genomes:

during stabilisation of synthetic oilseed rape
-> homeologous recombination plays a key role
-> its level depends on genotype, cytoplasm and gametes

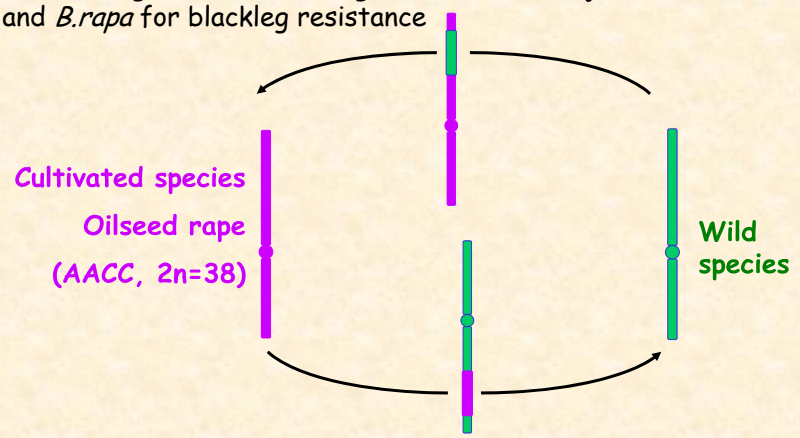
- Nature and frequency of structural rearrangements?
- Impact of functional regulation?

between A and C genomes in *B.napus*
-> control by *PrBn* and a network of genes
-> NRT are induced on different chromosomes

- Genes implied in homoeologous recombination control?
- Use of the genetic control to generate a diversity in *B.napus*

Use of results on homologous and homeologous control

Gene introgression from *B.nigra* (BB, 2n=16), *B.juncea* (AABB, 2n=36) and *B.rapa* for blackleg resistance



Gene flow from transgenic oilseed rape to wild radish

French polyploidy network (ANR)
How do these mechanisms arise at different time scales?

LONG TERM EVOLUTION, DIVERGENCE

(Years)

10 000

Triticum aestivum
(2n=6x=42)

B.Chalhoub et al. (INRA Evry)
J.Jahier et al. (INRA Rennes)

Nicotiana tabacum
(2n = 4x = 48)

M.A Grandbastien et al.
(INRA Versailles)

1000

Brassica napus
(2n = 4x = 38)

A.M.Chèvre et al. (INRA Rennes)
E.Jenczewski (INRA Versailles)
H.Thiellement, K.Alix (INRA Moulon)
B. Chalhoub et al. (INRA Evry)

100

Spartina anglica
(2n = 12x = 120)

M.Ainouche et al. (Rennes Univ.)

Synthetic allopolyploids

NEOPOLYPLOIDS

-> in project:
European network

2) Sequencing of the highly duplicated *B. napus* genome

(ANR: 2010-2013)

Boulos Chalhoub

Coordination with national efforts:
-French Brassica Network

Coordination with international efforts:
-Multinational *Brassica* Genome Project consortium
-EU coordination and collaboration (I. Bancroft, Guy Barker)
-US and Canada coordination and collaboration (C. Town, Chris Pires, Isobel Parkin)
B. Rapa and *B. oleracea*

Sequencing of a winter *B. napus cv.* (strategy and timeline)

Genome Sequencer FLX

Roche / 454

Sequencing (50X; Solexa) of 72 pb reads & pair-ends

20X (400) bp reads

1- Sequencing and sequence assembly Year 1 (2010)

2- Alignment to *B. rapa* and *B. oleracea* genome sequences Year 1 (2010)

Collaborations B. rapa cv Chiifu: 5X genome sequencing

B. oleracea cv B0001 5X genome sequencing

3- Anchoring on dense genetic maps: Years 1, 2 and 3

Dense genetic maps

Resequencing of 7-8 other genotypes (P1, P3: Régine Delourme P4: Brunel) (yerar 1, 2 and 3): Solexa paired ends

Illumina / Solexa Genetic Analyzer

(Parents of 4 segregating populations)

Develop and Map more than 10000 SNPs that will help in anchoring of sequence contigs on genetic maps as well as their wide use in oilseed rape breeding programs.

Rapeseed yield under nitrogen and water limitations
team

Alain Bouchereau
Nathalie Nési ; Françoise Le Cahérec

Objectives

Goals : Improvement of the energetic yield of rapeseed production

- ❖ Increasing oil production
- ❖ Decreasing nitrogen and water inputs
- ❖ Reducing energetic cost of oil extraction
- ❖ Preserving quality of co-products

Global objective :

To optimize oil yield under limited nitrogen and water regimes and to ensure co-products quality

Focus

Identification of genetic and physiological determinants of :

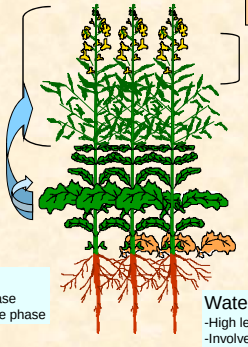
1. Yield

Yield potential
- architecture (undetermined development)
- yield production (compensations)

2. Seed and meal quality

Seed filling
- High oil content
- Negative correlation between oil and proteins

Meal digestibility
- Occurrence of antinutritional compounds



3. N use efficiency

Leaf senescence and N remobilization
- High efficiency of N absorption during the vegetative phase
- Low efficiency of N remobilization during the reproductive phase

4. N metabolism under low N inputs and low water availability

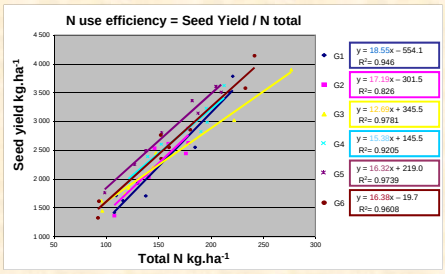
Water stress and N remobilization
-High levels of nitrogen compound accumulation under water stress
-Involvement of glutamate metabolism both for source osmoregulation and sink nutrition

❖ Improvement of sink/sources relationships
❖ Optimization of C/N ratio for oil production

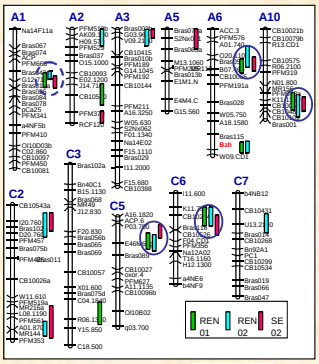
Genetic variability of yield, NUE and seed oil content

Genotype screening
under different N regimes

ARCOLE (06/08),
GENERGY (08/11)



Identification of genetic determinants
(QTLs) of seed oil content



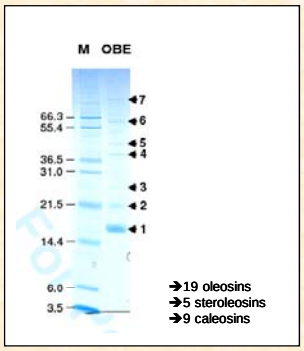
GOP-Q5 (01/04),
OSRCROP (06/08)

Delourme et al., TAG 2006

Seed and meal quality

Oil extractibility (APBV, CB, CETIOM)

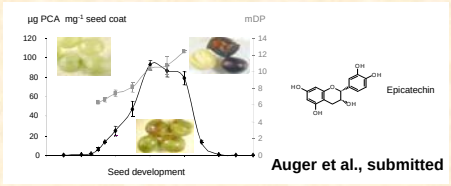
Proteomic description of oleosome composition in rapeseed



Jolivet et al., Proteomics 2009

Valorisation of co-products (APBV, URC, IJPB)

Identification of flavonoids in seed coats (LC-ESI-MS^n)



Auger et al., submitted

Cloning of candidate genes involved in seed flavonoid metabolism

Orthologues Bn*TT* (12 cibles)

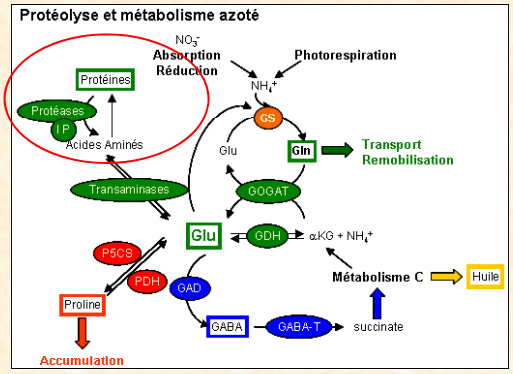
	Locus At*TT*	Produit	Gènes Bn*TT*
Part. Allemande	*TT1*	WIP zinc-finger family	4
	TT4	CHS	> 10
	TT5	CHI	4
	TT6	F3H	2
	TT7	F3'H	1
	TT3	DFR	2
Part. Française	*BAN*	ANR	4
	TT10	laccase	> 3
	TT12	MATE-transporter	2
	TT2	MYB transcription factor	2
	TT16	MADS transcription factor	2
	TT8	bHLH transcription factor	2

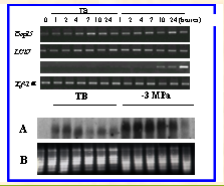
Nesi et al., Plant Cell Rep. 2009
Auger et al., Planta 2009

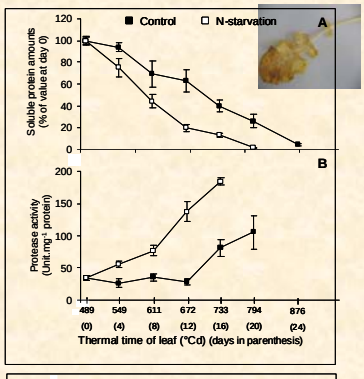
CompGen (04/05)

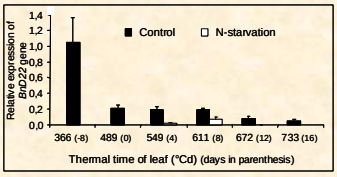
N remobilization during leaf senescence

Regulation of proteolysis (APBV, EVA)





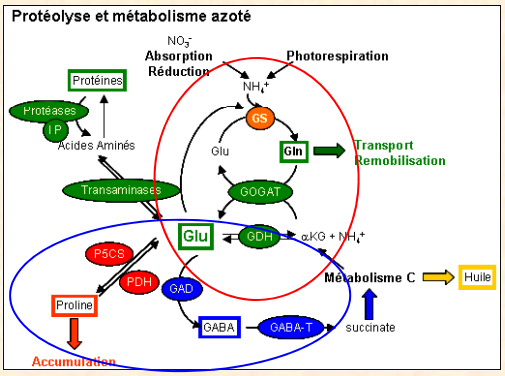


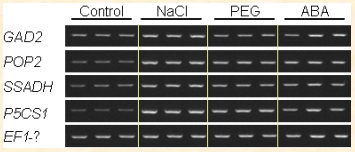


Desclos et al., Plant Physiol., 2008 ; Desclos et al., 2009, in prep.

N remobilization during leaf senescence

Regulation of genes involved in glutamate metabolism





Glutamine synthetase BnGS1 :

- 5 genes AtGS → more than 15 genes BnGS1 identified

Anova (p< 0.0001)

	Low N	Old leaves	Flowering time
BnGSR1-2_Y12459			-
BnGSR1-1_X76736		-	-
BnGSL2_Y12458		-	-
BnGSL1_X72751		-	-
BnGS1_5			
BnGS1_2		+	
BnGS1_1		+	Transient +
BnGS1_4	+	+	Transient +
BnGSR2-2_Y12460		+	
BnGSR2-1_X82997		+	
BnGS1_7	+		+
BnGS1_6	+		Transient +

➢Differential transcription during senescence

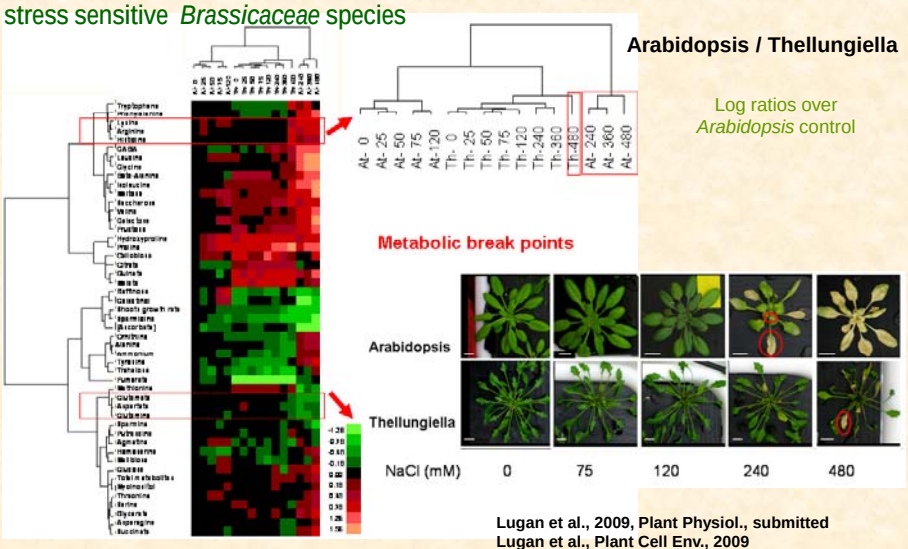
Biosynthesis and catabolism of proline

Biosynthesis and degradation of GABA

Renault et al., submitted

N remobilization during leaf senescence

Comparative metabolic profiling between water and nitrogen stress tolerant and stress sensitive *Brassicaceae* species



Lugan et al., 2009, Plant Physiol., submitted
Lugan et al., Plant Cell Env., 2009

Disease resistance team

Régine Delourme & Maria Manzanarès

► Main OSR diseases in France

Stem canker
Leptosphaeria maculans
anamorph *Phoma lingam*

Sclerotinia
Sclerotinia sclerotiorum

Light leaf spot
Pyrenopeziza brassicae

Powdery mildew
Erisiphe polygoni

Alternaria
Alternaria brassicae

Verticillium wilt
Verticillium longisporum

Clubroot
Plasmodiophora brassicae

Broomrape
Phelipanche ramosa

From CETIOM

► Main OSR diseases in France

Stem canker
Leptosphaeria maculans
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Phelipanche ramosa

From CETIOM

► Stem canker (*Leptospheria maculans*)

Leaf spots

- One of the most imortant disease worldwide (Europe, Canada, Australia)

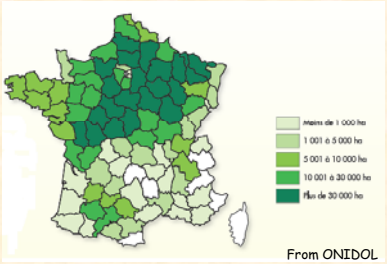
Crown necrosis

- **5 to 20 %** yield losses in France

(photo CETIOM)

- Disease incidence and severity could increase in relation with climatic changes (Evans et al., 2008)

Oilseed rape in France (2008)



From ONIDOL

Genetic resistance to *L. maculans*

- **Qualitative resistance: from seedling stage**

  ➡ in *B. napus*

  in related species
  (*B. rapa*, *B. juncea*, *B. nigra*)

  ⇄ many *Rlm* genes
  Specific: R-Avr inter.
  Easy to overcome

- **Quantitative resistance: at adult stage**

  ➡ in *B. napus* (Jet Neuf…)

  ⇄ Polygenic: many QTL
  Partial, not specific?
  Durable

Durable management of blackleg resistance

What are & how to associate resistance factors for a durable management of resistance faced to a highly variable pathogen population ?

- Organisation of the resistance genetic factors ? Relation specific resistance genes/ quantitative resistance ?
- Evolution of pathogen populations depending on the type of resistance used ?

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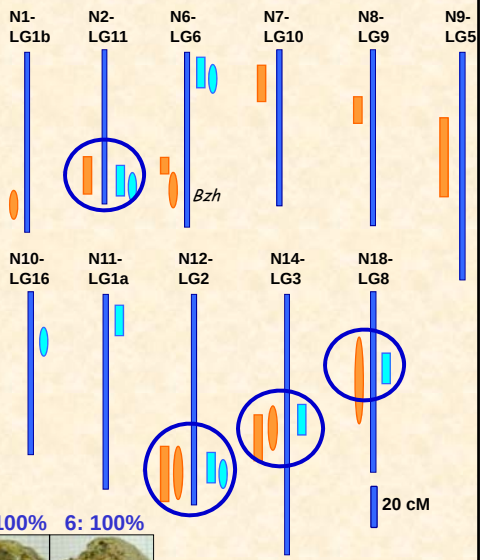
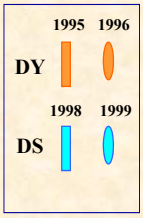
Genetic analysis of the quantitative resistance



Resistance QTL : two progenies

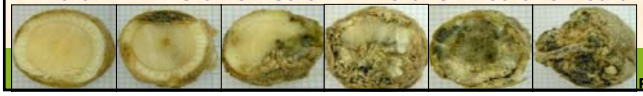
Darmor-bzh x Yudal
Darmor x Samourai

(Pilet et al, 1998, 2001)



Classes G2 index

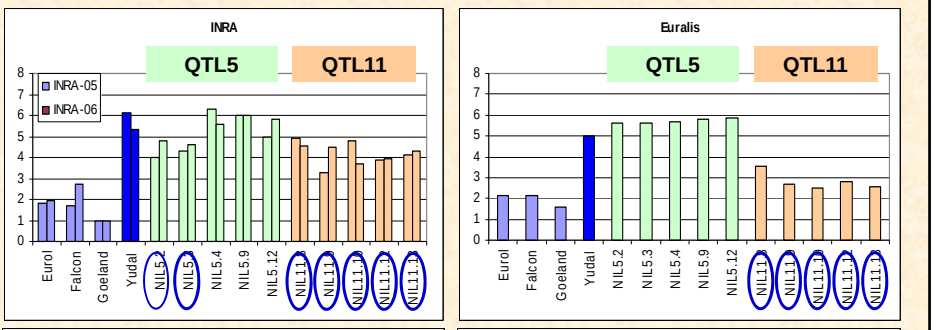
1: 0% 2: <25% 3: <50% 4: <75% 5: <100% 6: 100%



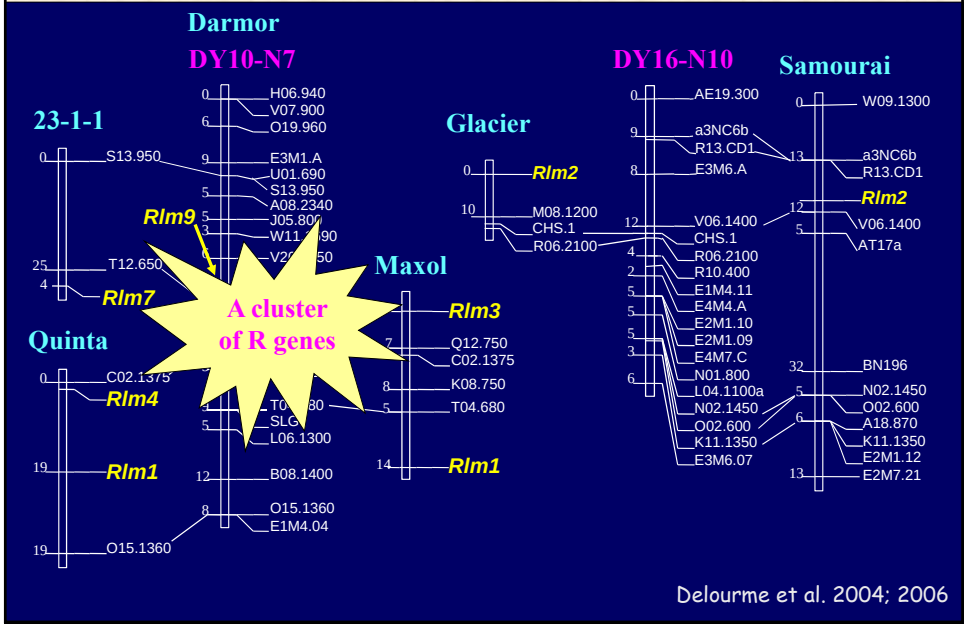
Photos JN Aubertot

Trials in H2005-06: G2 index

(Delourme et al, 2008)

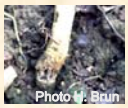


Localization of *B. napus Rlm* genes



Localization of *Rlm* genes / QTL for adult resistance (DI)

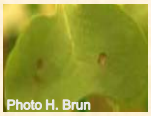
Quantitative resistance



QTL

Specific resistance genes

* From oilseed rape
(*Delourme et al.2006*)



* From related species

B.nigra: Rlm10
(Chèvre et al 1996)

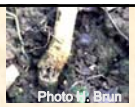
B.juncea: Rlm6
(Chèvre et al. 1997)

B.rapa: Rlm1, 7
(Leflon et al 2007)

Durable management of blackleg resistance

What are & how to associate resistance factors for a durable management of resistance faced to a highly variable pathogen population ?

- Organisation of the resistance genetic factors ? Relation specific resistance genes/ quantitative resistance ?
- Evolution of pathogen populations depending on the type of resistance used ?



What about durability?

Overcoming under real cropping conditions (3 years) of :

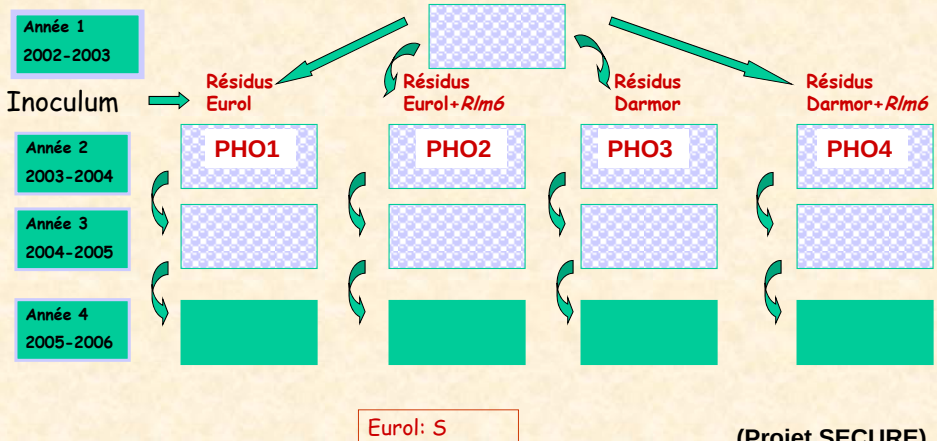
- *Rlm1,* a *B.napus* specific gene in France *(Rouxel et al 2003)*
- *LepR3,* a *B.rapa* specific gene in Australia *(Li et al 2003)*

Experimental design to assess potential durability *(Brun et al. 2000)*

Recurrent Selection in the field

(Brun et al 2000)

Local Inoculum : Oilseed rape residues



Eurol: S
Darmor: RQ

(Projet SECURE)

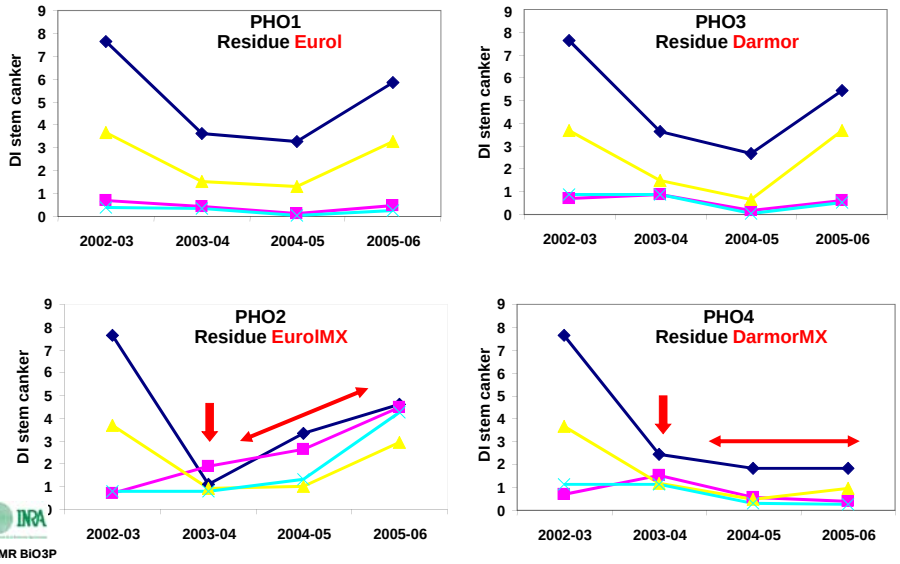
Effect of genetic background?

Stem base Canker over years

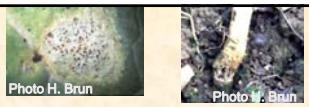
Eurol
EurolMX
Darmor
DarmorMX

(Brun et al, 2007)

MX = Rlm6



UMR BIO3P



What about durability?

Overcoming under real cropping conditions (3 years) of :

- *Rlm1,* a *B.napus* specific gene in France (Rouxel et al 2003)
- *LepR3,* a *B.rapa* specific gene in Australia (Li et al 2003)

Experimental design to assess potential durability *(Brun et al. 2000)*

- The results of the potential durability obtained experimentally for *Rlm6* were close to the results of the overcoming of *Rlm1 and LepR3* resistances under commercial deployment
- Association of quantitative and qualitative resistance improved potential durability

In progress… Strategies for Management in time and space

New targets for the future?

- Heterosis
- Multi-trait resistance
- Insect resistance?

THANK YOU

Conventional

Cleistogamous