

The use of genomic tools for the characterization of a Quantitative Resistance to Downy Mildew (*Plasmopara halstedii*) in cultivated Sunflower, *Helianthus annuus*

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The French Plant Genomic Resource Center

The CNRGV has been created in France in 2004 by the French National Institute for Agricultural Research (INRA). It's a **non-profit center** dedicated to the scientific community. It is both a **repository centre for plant genomic resources** (such as BAC and cDNA libraries) and a **service provider**. **More than 100 genomic libraries from model and crop species are currently available**. We carry out services such as BAC library construction, that can be associated to our efficient 3D-pools production and screening methods.

Our processes are certified under ISO9001: 2000. Any exchanges of resources is ruled by Material Transfer Agreement

CNRGV is involved in numerous projects on various model and crop species including :

- Wheat and barley improvement: TriticeaeGenome FP7 (1)
- Tomato: Physical mapping for sequencing of chromosome 7 (2)
- Sunflower: SunyFuel et Mildew (3)
- Medicago: Various projects as Medicago Stock Center (4)
- Eucalyptus : Eucalyptus BAC libraries as tools to identify and characterize genomic sequences involved in wood formation (5)
- Pepper : PHYTOSOL-2 Functional confirmation of candidate genes for a broad-spectrum resistance QTL against Phytophthora in Solanaceae (6)
- Rapeseed improvement (7)
- Chicory Genetic and physical mapping of the S-locus (Asteraceae) (8)
- Sugarcane genome sequencing (9)

CNRGV's collaborators:

- (1) ETGI / ITM / Triticeae Genome partners
- (2) Génétique, Diversité et Ecophysiologie des Céréales UMR 1095 Clermont-Ferrand - France
- (3) Laboratoire de génétique et d'amélioration des fruits INRA/INP-ENSAT Toulouse - France
- (4) Laboratoire des Interactions Plantes Micro-organismes INRA/CNRS Toulouse - France
- (5) Centro das Florestas e Produtos Florestais, Lisbonne - Portugal
- (6) Caractérisation Fonctionnelle des interactions Plantes - Bioagresseurs INRA-UR1052-GAFL Avignon - France
- (7) The Australian National University, Canberra - Australia
- (8) Amélioration des Plantes et Biotechnologies Végétales UMR118 INRA-AgroCampusOuest-UniversitéRennes - France
- (9) Stress abiotiques et différenciation des végétaux cultivés, UMR USTL/INRA1281/SADV Lille - France
- (10) Departamento de Botânica-IBUSP São Paulo, Brasil
- (11) Structure et évolution des génomes CIRAD, UMR 1098 Montpellier, France

The Promosol Mildew project

Here we focus on the collaborative Promosol Mildew project which aims to explain durable resistance to downy mildew in Sunflower.

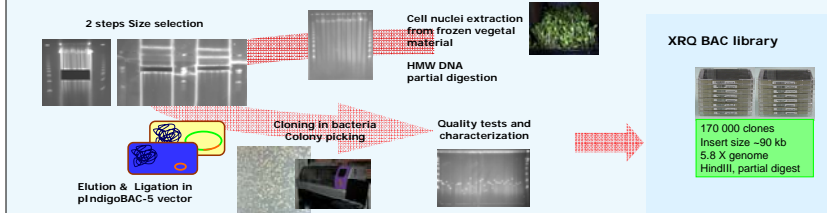
Laboratoire Interactions Plantes/Microorganismes, UMR 441-2594 INRA-CNRS, Castanet Tolosan
 UMR 1095 Amélioration et Santé des Plantes INRA/ Université Blaise Pascal, Clermont Ferrand
 UMR 1065 Santé des plantes INRA-ENITA, Bordeaux
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Downy mildew is one of the potentially most dangerous diseases of sunflower in Europe. Total resistance genes have already been used in breeding and overcome by this obligatory parasite oomycete. But a major QTL « Horizon » conferring quantitative resistance to race 710 has also been detected in 2005. This quantitative resistance to sunflower Downy Mildew was further studied on a larger population of recombinant inbred lines (RIL XRO resistant * PSC8 susceptible) not carrying efficient major resistance gene, in fields naturally infested by the pathogen. **The major quantitative trait locus (QTL) localized on linkage group 10 has proven to explain almost 40% of variation** (*Tourville et al., 2008 **Year et al, 2008), and is not linked to any of the known race-specific resistance genes called Pi genes. **Its support interval is 5 cM long**. The resistant phenotypes of the recombinant plants are currently being evaluated in order to restrict the QTL support interval.

Below is described how CNRGV genomic tools are being used to progress toward a physical map linked to an enriched genetic map, as a first step to the positional cloning of the QTL.

CNRGV Dedicated Genomic tools

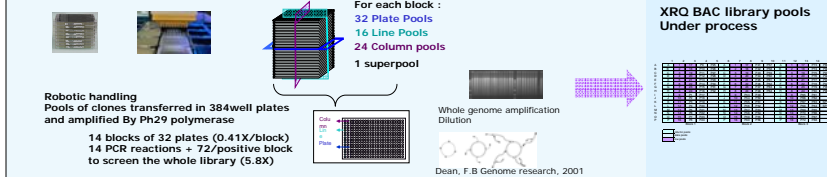
BAC library construction on resistant XRO genotype:



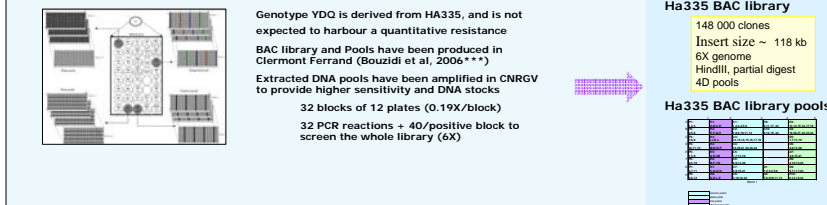
Macroarray production on XRO genotype library



Three dimensional pools (3D-pools) production on XRO genotype library



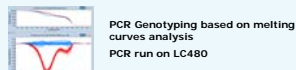
Four dimensional pools (4D-pools) amplification on HA335 genotype library (ii):



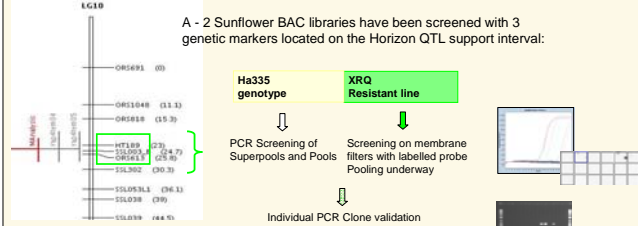
BAC Fingerprinting



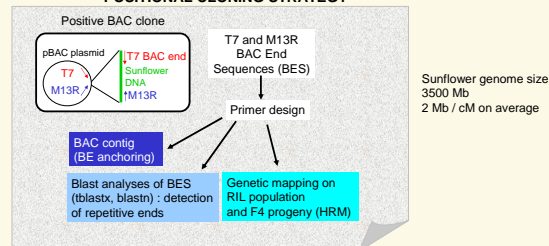
Screening of recombinant lines by HRM:



Horizon QTL fine mapping : towards a BAC contig

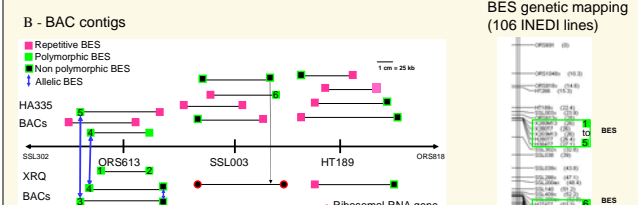


POSITIONAL CLONING STRATEGY



16 BACs have been identified with genetic markers, and their ends have been sequenced. The polymorphic BES have been mapped with the 106 available RIL lines, very close but after ORS613 marker, probably because of lack of recombination events in the region.

LIPM laboratory is currently mapping 1774 F4 lines chosen to be recombinant in F3 in the ORS613 - HT189 region with the polymorphic BES markers in order to restrict the QTL interval, and their phenotyping is in progress.



New BAC screenings are being processed with the BES derived - markers to extend BAC contigs in both genotypes with the aim to produce new genetic markers and enrich the genetic map.

Throughout this process, we aim to cover the whole interval where the QTL is genetically present with overlapping BACs. The sequencing of the MTP of these BACs and their annotation will further lead to identification of candidate genes for resistance to downy mildew.

The libraries, tools and techniques presented above as well as similar tools produced on numerous plant species are available for scientific community. See more information and make your own request for specific genomic tool or service - or set up of collaborative project - on our web site :

<http://cnrgv.toulouse.inra.fr/>

Aknowledgments

- [i] This project is partially supported by PROMOSOL (French association for the promotion of oil crops: CETIOM, ONIDOL,OLEOSEM)
- [ii] Genoplante BAC library