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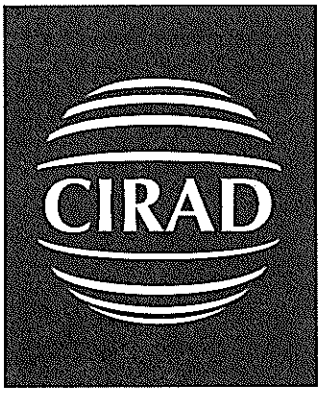


Programme Canne à sucre / Biotrop

# "Fine mapping of a major Rust Resistance Gene in Cultivar R570 with a view towards it cloning through map-based chromosome walking"

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Report on the project :

# "Fine mapping of a major Rust Resistance Gene in Cultivar R570 with a view towards it cloning through map-based chromosome walking"

**Organization :** CIRAD, Montpellier, France

**Period :** March, 1997 - August, 1999

**Principal investigators :** Dr. Angélique D'Hont, Dr. Claudia Kaye and
Dr. Jean Christophe Glaszmann

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## Background

A major gene for rust resistance has been identified in cultivar R570 (Daugrois *et al*, 1996).

Rust disease is caused by the fungus *Puccinia malenocephala* Syd P. Syd and is distributed worldwide. Inheritance of resistance to rust was investigated in the self progeny of cultivar R570, which was also used to build the RFLP genetic map of this cultivar (Grivet *et al*, 1996). Resistance was evaluated through both field and controled greenhouse trials. The results demonstrated that the field scores are very well correlated between plant cane and first ratoon and in very good agreement with the greenhouse artificial inoculation tests. A clear-cut 3 (resistance) : 1 (susceptible) segregation indicative of a dominant resistance gene was observed. RFLP mapping indicated that this gene was linked at approximately 10 cM to a marker revealed by the cDNA clone CDSR 29. This marker was not linked to any of the cosegregation groups of the R570 map (Grivet *et al*, 1996).

This gene is the first major gene identified in sugarcane. ICSB was thus interested in using it to test the feasability of map-based cloning in the complex aneuploid polyploid that sugarcane is. For this purpose the BAC library developed by R.Wing and co-workers was constructed using cultivar R570 (Tomkins *et al*, 1999).

The first step for map-based cloning is to develop a fine map in the vicinity of the gene ; this was the objective of the present project. For this purpose, we developed and characterized for rust resistance large selfed progenies of R570 and we followed two strategies to find markers closely surrounding the rust resistance gene ; one is based on the synteny and colinearity demonstrated between Graminae species and the second is based on Bulked Segregant Analysis (BSA) with AFLP markers. We also tested the pathogenicity variability of the fungus.

## Development and characterization of plant material resources for fine mapping

The 3:1 segregation indicating the presence of a major resistance gene was first demonstrated with 150 individuals from a self progeny of R570. We have now confirmed this result on an additional 700 individuals from another self progeny of R570 which was characterized for rust resistance, two years, in a randomized complete block layout with three to five replications. The susceptibility level of each plot was graded on a 1 to 9 scale. The clone with a susceptibility level > 2 are considered as susceptible. Furthermore, segregation of the resistance gene in progenies from 3 biparental crosses between R570 and susceptible cultivars (comprising 300, 600 et 800 clones respectively) has been analyzed and revealed the expected 1:1 ratio for a major gene. More recently, one thousand and four hundred additional clones has

been produced and characterized for rust resistance. A total of 2100 clones characterized for rust resistance are thus now available.

**Localization of the gene at the extremity of linkage group VII by use of synteny with sorghum, maize and rice**

The first strategy developed to find markers in the vicinity of the resistance gene takes advantage of the synteny between sugarcane, sorghum, rice and maize. We first positioned the CDSR 29 probe on our sorghum map. It is located at the extremity of the sorghum linkage group D of the map of Dufour *et al*, 1997. Then, through comparative genome mapping, we identified homoeologous segments on the available sorghum, rice and maize maps. The homoeologous segments are located on rice chromosome 2 and maize chromosomes IV and V. We selected 184 potentially useful probes in the targeted regions. Eighty six, 66 and 32 probes were chosen on the basis of the maize, rice and sorghum maps available, respectively. These included 87 maize probes, 75 rice probes, 9 sorghum probes and 13 probes from oat and barley. These heterologous probes were evaluated by southern blot hybridization for their ability to cross-hybridize with sugarcane DNA. One hundred and six probes produced a usable RFLP profile and were analyzed on a set of 88 individuals of the R570 population. One thousand and twenty markers were generated out of which 524 were polymorphic and 311 segregated 3:1. Thirty two of these 311 single dose markers were redundant. Finally, 279 markers were mapped. This enabled us to position the rust resistance gene on linkage group VII of the R570 map and to locate an additional 122 markers corresponding 53 probes on this linkage group. The other markers were either unlinked (30), linked to isolated markers (32) or assigned to other linkage groups (95) : group I, 11 markers ; group II, 12 markers ; group IV, 4 markers ; group V, 4 markers ; group VIII, 10 markers ; group IX, 36 markers ; group X, 13 markers ; group XI, 5 markers.

Sixty eight %, 65% and 42% of the probes chosen based on sorghum, maize and rice maps, respectively, hybridized with sugarcane DNA out of which 77%, 39% and 50%, respectively, were mapped on linkage groups VII. The percentage of hybridization reflects the divergence at the sequence level, whereas the percentage of markers mapped on group VII reflects the degree of synteny and colinearity between these species. Sorghum appears the most informative species to help saturate sugarcane maps. However, the published sorghum maps are less saturated than the ones of maize and rice and thus fewer sorghum probes are available. The percentage of maize probes that hybridized with sugarcane DNA is higher than the rice one. However, the maize genome has undergone numerous rearrangements and thus breaks of colinearity and synteny are more frequent between sugarcane and maize than between sugarcane and rice.

The resistance gene was mapped, as well as 19 markers, to one of the 17 cosegregation groups belonging to linkage group VII. All the markers were located on only one side of the rust resistance gene, which thus appeared at a terminal position. The closest RFLP marker remained the one revealed with CDSR29, which fully cosegregated with one marker revealed by the maize probe ISU 134 and one marker revealed by the rice probe C673.

This first strategy thus allowed us to locate the gene on linkage group VII but did not provide any marker closer to the gene or located on the other side of the gene. This can be explained by the fact that the gene is located at the extremity of a chromosome and the homeologous segment on sorghum, rice and maize linkage IV are also located at the extremity of chromosomes. The homeologous segment on maize linkage group V is located at a central position but is adjacent to a break of synteny with sugarcane.

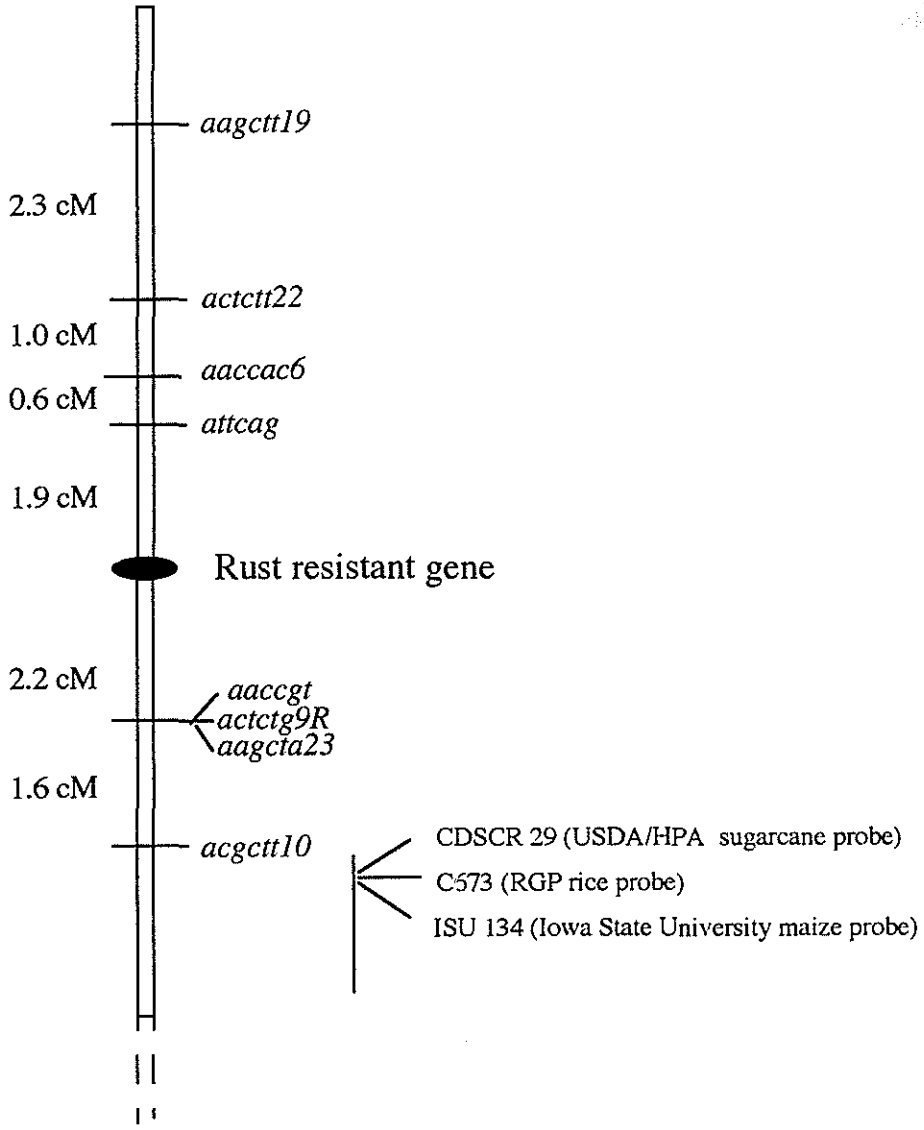
**Development of a fine map around the gene using Bulked Segregant Analysis and AFLP markers**

The second approach is to use bulked segregant analysis (BSA) with AFLP markers. AFLP allows for a large number of markers to be rapidly screened with BSA in order to identify markers located in the region of the rust resistance. Two pools of DNA were made, one with DNA from 5 individuals resistant to rust and the other from 5 individuals susceptible to rust, all from the selfed progeny of R570. These pools were screened with the 64 AFLP primer combinations available in the commercial Kit from GibcoBRL and revealed 6 linked with the rust resistance gene. These markers were ordered and the distance between them estimated based on the analysis of 316 individuals from the self progeny of R570 using the Haldane mapping function. Three of these markers are located on one side of the rust resistance gene : two cosegregated totally with one another and were located at 2.2 cM, and one was located at 3.8 cM. The other three markers were located on the opposite side at 2.5 cM, 3.5 cM and 5.8 cM of the gene. Twenty six individuals recombinant for at least one of these markers were identified to be used for further mapping of new candidate markers identified by BSA.

The commercial AFLP kit consists of 64 primer combinations only. To continue this strategy, we designed additional AFLP primers. New bulks were also created, two by pooling susceptible individuals, two by pooling resistant individuals and one which consisted of susceptible recombinant individuals (three individuals recombinant for each side of the gene). The susceptible recombinant bulk was created in order to identify markers that are more closely linked to the rust resistance gene ; markers absent from this pool, absent in the pool of susceptible individuals but present in the pools of resistant individuals should more closely linked to the rust resistance gene.

We are facing important difficulties with the new primers, that we are buying from various suppliers. The quality of the primer is very variable and leads to poor amplification. This is particulary critical for the analysis of a high polyploid such as sugarcane since this leads to profiles missing the single dose bands, which are the bands we are interrested in. The 250 primer combinations tested so far, for which we got a profile of good quality, allowed us to identify two new markers located at 2.2 and 1.9 cM on both sides of the gene.

The current map encompasses 8 AFLP markers in an interval of 10 cM, the closest being located at 1.9 cM and 2.2 cM on both sides of the gene.




**Screening of the BAC library with the closest markers**

Three of the closest AFLP markers (aaccac6, actctg9R and attcag) surrounding the rust resistance gene were cloned and used to screen the BAC library of R570 constructed by R. Wing and co-workers. These markers hybridized to 22, 5 and 14 BACs, respectively, which represent BACs containing the various allelic versions of the cloned AFLP markers. These BACs were analyzed by AFLP in order to determine the ones which dispay the markers linked

to the resistance gene. This led to the identification of two BACs for each of the 3 cloned AFLP markers. The library was also screened with CDSR29 by Wing and co-workers ; this probe hybridized to 13 BACs from which 2 encompassed the allelic version of the marker linked to the resistance gene.

The BACs corresponding to the AFLP marker aaccac6 have been fingerprinted and end cloned. These end clones are now available to be used to start building a contig and determine the relationship between genetic and physical distances in this area of the sugarcane genome.

One of the cloned AFLP markers (aaccac6) located on the distal side of the gene was mapped on sorghum. Since the cloned AFLP marker used as a probe did not provide an RFLP profile of good enough quality, we used a sub-clone of one of the corresponding BACs as probe. This clone was mapped at the expected syntenic position which is the extremity of our sorghum group D. This confirmed that we did not find RFLP markers on the other side of the gene because the gene is located at a terminal position on the chromosome rather than because there is a break of synteny between sorghum and sugarcane in this genome region.

## Variation of the pathogenicity of the fungus

In parallel, to the present project we were interested in testing the efficiency of the resistance gene identified in R570 against various rust isolates of the fungus *Puccinia melanocephala* collected in various countries around the world. This in order to test if a variation of the pathogenicity of the fungus exists which could affect the efficiency of the gene. For this purpose, we inoculated detached leaves with rust suspensions of the various isolates. They consist of 7 isolates from Reunion (Indian Ocean island), Brazil, Zimbabwe, Florida (USA), Colombia and Guadeloupe (West Indies island). The gene appears efficient against all the isolates tested so far.

## Conclusion and prospects

We have developed and characterised the plant material resources necessary for fine mapping and have identified 8 AFLP markers in an interval of 10 cM on both sides of the rust resistance gene. However, we are still at 1.9 cM and 2.2 cM from the gene, whereas the objectives were to be inside 1 cM on both sides.

The strategy based on synteny with Graminae was not as efficient as expected due to the terminal position of the gene on the chromosome. This position could not be anticipated before doing the mapping work.

The strategy using BSA and AFLP markers is efficient in providing markers closer on both sides of the gene. However, we progressed slower than expected due to the high polyploidy of sugarcane which, amplifies the consequence of any technical difficulty, such as the one due to the variable quality of the commercial primers.

The rice BAC library developed by Wing *et al* represent an attractive additionnal/alternative resource to assist fine mapping, then chromosome walking, toward the rust resistance gene : the size of one genome of rice is half the size of one genome of sugarcane ; rice and sugarcane share important synteny and colinearity, and ; the rice BAC library ordering is currently being completed. One of the closest cloned AFLP markers (actctg9R) was hybridized on filter blots containing the Rice BAC library and hybridized to 17 BACs. The closest AFLP markers on the other side of the gene could also be hybridized likewise. The rice BACs that hybridized with the closest markers surrounding the rust resistance gene could then be used by Wing and co-workers in order to identify rice contigs homeologous to the target sugarcane segment. This would provide numerous markers to be tested and mapped on sugarcane and used to shorten the walk toward the resistance gene.

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