PROGRESS IN GENETIC MAPPING OF SUGARCANE SMUT RESISTANCE

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Abstract

A Quantitative Trait Locus (QTL) mapping study is under way to analyse the genetic determinism underlying sugarcane smut resistance. A total of 1295 polymorphic AFLP markers have been generated on a population of 200 individuals derived from a cross between R 570 (resistant) and MQ 76/53 (highly susceptible). This population is under evaluation for its resistance to smut in the field over three successive crop cycles and in two different locations on Reunion island. Inoculation was performed by dipping three-bud cuttings in a spore suspension, and susceptible clones were regularly interspersed in the field to trigger the epidemy. Different inoculation methods were also compared in greenhouse trials. The bud puncture method appeared the most efficient and will be used for further greenhouse evaluations of the progeny. Detection of marker-trait associations has been performed and so far has indicated a complex determinism for smut resistance. Many markers are involved with little effects. Nevertheless, among other traits that were looked at, two putative major genes originating from MQ 76/53 have been tagged: a gene involved in the red color of the internode and a rust resistance gene. Locus specific markers, SSR markers from sugarcane and maize and sequences differentially expressed in response to challenge by smut (provided by the South African Sugar Association Experiment Station), will be mapped. This should help explain the genetic variation in smut resistance observed in the trials.

Keywords: sugarcane, smut, *Ustilago*, QTL, disease resistance, AFLP

Introduction

Smut caused by *Ustilago scitaminea* can be responsible for serious yield losses. The disease can be controlled by planting resistant cultivars, but their selection requires long term field trials. Understanding the genetic mechanism underlying varietal resistance to sugarcane smut is a prerequisite in designing more efficient breeding strategies. A QTL mapping project, initially presented at the 75th Congress of the South African Sugar Technologists' Association in 2001 (Raboin *et al*., 2001), has therefore been undertaken to dissect the quantitative resistance of sugarcane cultivar R 570 to smut.

This paper reports progress made towards the construction of the genetic map in a cross between cultivars R 570 and MQ 76/53, and the first insight obtained on the genetic determinism of smut resistance. Interesting side results concerning other traits are also presented.

Material and methods

Smut resistance characterisation in the segregating population

A bi-parental cross was studied involving the resistant cultivar R 570 (H 32/8560 x R445) and MQ 76/53 (Trojan x SES 528), a highly susceptible cultivar. R 570 was used as a female after emasculation by hot water treatment. A preliminary experiment with one hundred clones was planted in November 1998 at the CERF-le Gol station, using a randomised block design with two replicates. A second trial was planted in September 2000 at the Ligne Paradis CIRAD station with three replicates. A total of 196 clones were studied, including the 100 used in the preliminary experiment. In this trial, smut susceptible clones have been regularly interspersed to facilitate the epidemic. In both trials, basic plots consisted of 2.5 m rows planted with eight inoculated cuttings.

Sugarcane was inoculated with smut by dipping three-bud cuttings for 20 minutes in a spore suspension of $5x10^6$ spores per ml. Viability of spores was previously checked on agarose in Petri dishes. Inoculated cuttings were incubated for 24 hours under high humidity before planting. Smut incidence was measured by counting newly emerged whips every 15 days, and the number of whips was summed over the entire crop cycle. Old whips were marked with paint to avoid counting them twice. This assessment was performed in plant cane and two ratoons for the preliminary trial (P1, P2, P3) and plant cane and one ratoon for the Ligne Paradis trial (LP1, LP2). Besides smut evaluation, we measured various traits such as resistance to rust caused by *Puccinia melanocephala*, number of millable stalks, brix, stalk diameter, stalk colour and number of flowers. Rust resistance was assessed on a scale of 1-9 (Tai *et al.*, 1981). Brix was measured with a hand refractometer using six individual millable stalks randomly chosen per plot. The number of millable stalks was counted over the entire plot.

Comparison of different inoculation methods

Different inoculation methods were compared in greenhouse trials for resistant cultivar R 570, susceptible cultivar B34/104 and highly susceptible cultivar MQ 76/53. From 12 to 48 one-bud cuttings were inoculated for each variety x treatment combination. Inoculation was performed by injection of a spore suspension ($5x10^6$ spores/ml) in one week old germinating shoots, by dipping the cuttings in a spore suspension as described above, or by puncturing the buds with a needle previously covered with smut spores.

AFLP genotyping and mapping

The analysis of 38 AFLP primer combinations (GIBCO BRL AFLP Kit genome I) produced 1295 polymorphic markers on 196 clones of R 570 x MQ 76/53 progeny. The linkage relationships of simplex markers in the coupling phase were determined using Mapmaker (Lander *et al*., 1987). Two-point analyses were performed at a LOD score threshold of 5, and a recombination fraction threshold of 0.30. At this stage of the project, we did not perform the time-consuming multipoint analysis to order markers within the identified cosegregation groups because additional markers will be produced later on (microsatellites and RFLPs). Of these 38 primer combinations, 34 have already been used to build an AFLP map for R 570 (Hoarau *et al.*, 2001). Therefore, most of the bands specifically inherited from R 570 in the biparental population could be labelled in accordance with the name already defined in the published map.

Statistical analysis
Broad sense heritability was calculated at the experimental design level as follows:

$$H_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_e^2 / 2)}$$

Genetic (σ_g^2) and error (σ_e^2) variance estimates were obtained with the SAS VARCOMP procedure (Anon, 1990).

Marker-trait linkages were detected by performing one-way ANOVA for each marker. Only associations significant at $P<0.005$ were retained. An empirical threshold has been calculated according to Churchill and Doerge (1994). Empirical threshold values are obtained by permutating the trait data with respect to the genetic marker data. Ten thousand permutations were performed to determine the experimental threshold.

Results and discussion

Smut resistance characterisation in the segregating population
The variation observed in the two field trials for smut resistance, measured as the number of whips produced per plot, is quantitative. The two parents are positioned at each extremity of the distribution tails (R 570 being one of the most resistant clones under evaluation, and MQ 76/53 the most susceptible), suggesting a rather additive inheritance of sugarcane smut resistance in the studied cross. The accuracy of the evaluation increased with each crop cycle. Indeed, the broad sense heritability for the number of whips per plot was 0.26 in plant cane of the preliminary trial, 0.67 in the first ratoon and 0.7 in the second ratoon. In the Ligne Paradis field trial, broad sense heritability was 0.68 in plant cane and 0.75 in the first ratoon. Interesting results are therefore expected from the second ratoon in this trial.

Comparison of different inoculation methods
Previous screening of progenies with the dipping method in the greenhouse trials was not satisfactory. Disease incidence (number of one-bud cuttings presenting a whip/total number of one-bud cuttings inoculated) was very low. Different methods of inoculation were therefore also tested in the greenhouse trials using one-bud cuttings: injection, bud puncture or simple dipping. Results are presented in Figure 1. Injection and bud puncture are the two methods that distinguish more clearly the susceptible from the resistant clone. Being the easier of the two to use, the bud puncture method will thus be used for future greenhouse screenings.

First results arising from AFLP mapping

Description of the map
A total of 1295 polymorphic markers have been produced, of which 752 are simplex markers specific to one of the two parents (segregating in a 1 present : 1 absent ratio). Fifty-six per cent (421) of the markers originate from MQ 76/53, and 44% (331) originate from R 570. These markers have been gathered into 145 cosegregation groups (74 specific to R 570 and 71 specific to MQ 76/53). Of the 331 R 570 markers, 252 (76%) have already been mapped by Hoarau *et al*. (2001).

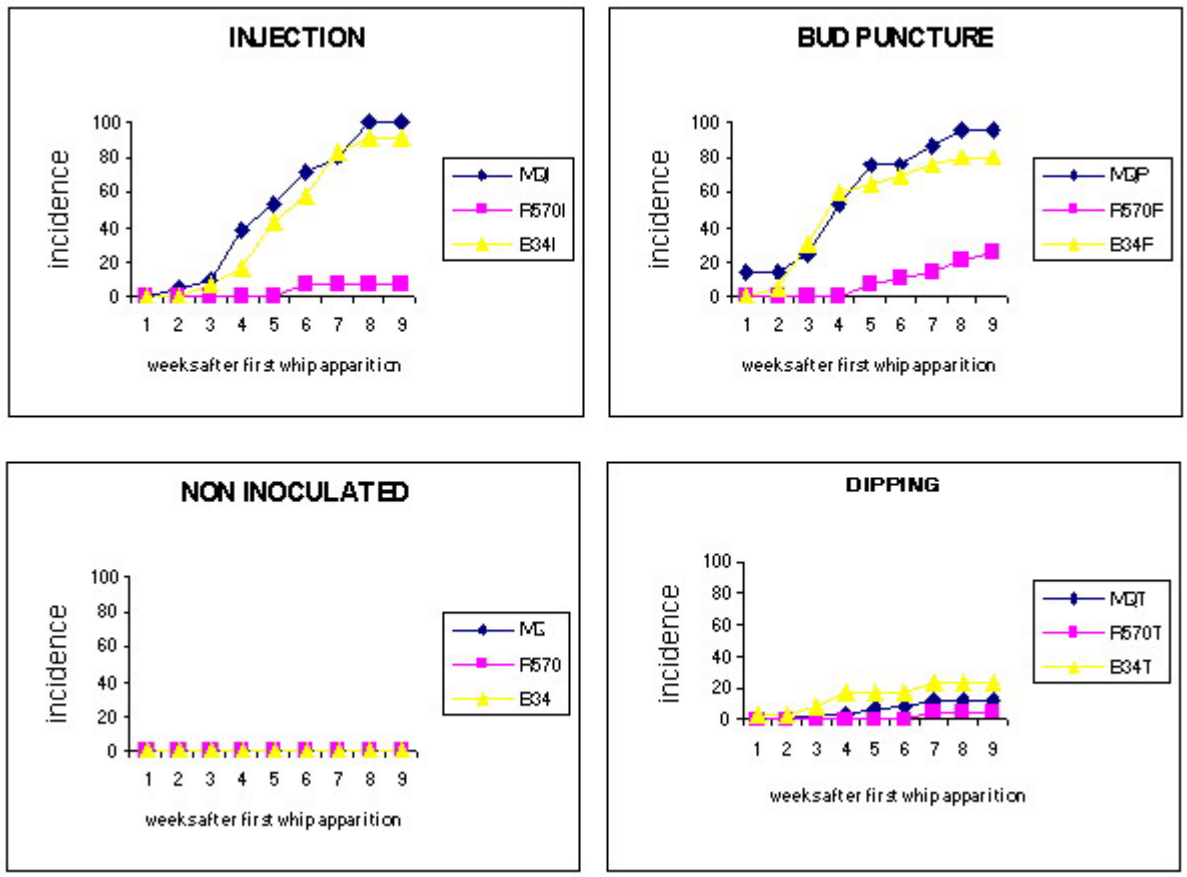

Figure 1. Comparison of different smut inoculation methods used in a greenhouse trial.

Tagging of two putative major genes

A 1:1 segregation ratio was observed for stem colour in the studied progeny. Half of the clones showed red coloured stems and the other half non-red stems (Figure 2). This finding suggests the presence of a putative major gene. The morphological trait coded as a simplex marker cosegregates with two MQ 76-53 specific markers in a small cosegregation group (Figure 3).

Figure 2. A red coloured stalk clone and a non-red stalk clone from R 570 x MQ 76/53 progeny.

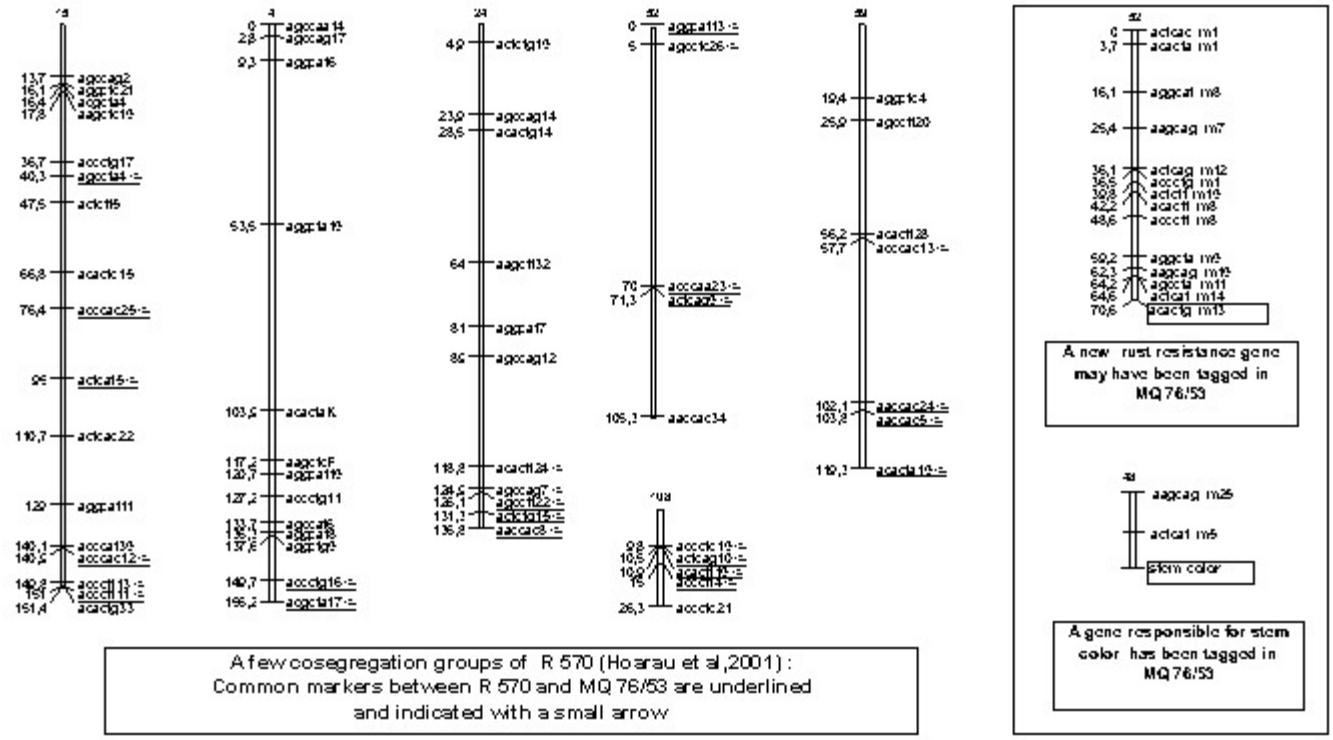

Figure 3. First results from AFLP mapping of a R 570 x MQ 76/53 cross.

A clear 7 resistant: 1 susceptible segregation ratio was observed for rust resistance. This result suggests that three major resistance genes may segregate in the progeny. As it is known that one of these genes came from R 570 (Daugrois *et al*., 1996), the other two are assumed to be brought by MQ 76/53. A strong association has been detected between rust resistance and a marker from MQ 76/53 in cosegregation group 52 (see Table 1). This suggests that we have tagged at least one of the two putative major rust resistance genes of MQ 76/53 (Figure 3). As expected, the already known markers linked to the R 570 rust resistance gene have also been detected in the present context. The two cosegregation groups (the one carrying the R570 rust resistance gene and the one carrying the putative MQ 76/53 rust resistance gene) have different haplotypes suggesting that, if they belong to the same homology group, they may carry different genes (alleles).

Localisation of common markers between R 570 and MQ 76/53

Most of the common markers between R 570 and MQ 76/53 in this study had already been mapped by Hoarau *et al*. (2001). Highlighting these common markers on the AFLP reference map reveals their distribution in clusters, suggesting that fragments of homologous cosegregation groups (i.e. chromosomes) may have the same haplotype in R 570 and MQ 76/53 (Figure 3). This could be a representation of linkage disequilibrium in sugarcane. R 570 and MQ 76/53 have no known common ancestors in their pedigree, but sugarcane cultivars in general have passed through the foundation bottleneck of the first few interspecific crosses between *S. spontaneum* and *S. officinarum* from which breeding programmes worldwide have been built. Linkage disequilibrium is therefore expected to extend over long distances (Stumpf, 2002).

QTL detection at P=0.005 (Table 1)

At this stage, a total of 128 QTAs have been detected for the six studied traits, representing 18 variables when considering only one QTA per CG or unlinked marker.

The number of QTAs found per variable ranges from 3 to 13. The individual effect of significant QTAs varies from 3 to 12% of the total phenotypic variation.

For the number of whips per plot, detected QTAs are not consistent between the preliminary and Ligne Paradis trials, since only one marker (acactt_r18) was detected in both trials (LP2, P3). Moreover, none of the detected QTAs explain more than 6% of the observed phenotypic variation. Smut resistance characterisation of the progeny will be improved by future greenhouse screenings and future ratoons of the field trial, and should enable a better understanding of the genetic control of resistance.

QTA detection has been performed for the other traits observed in the progeny. Two interesting markers for Brix have been detected consistently over three environments (LP1, LP2, P2). QTAs have also been detected for stalk diameter, stalk number and flowering. Special attention will be paid to flowering in future evaluations, because this trait presents a wide segregation and a high broad-sense heritability (0.88 in LP2).

Future developments

Smut resistance characterisation
Smut resistance evaluation of the 196 clones from R 570 x MQ 76/53 progeny will be further investigated. A new field trial (a replicate of the LP trial, using the same method of inoculation by dipping) was planted at the CERF-Le Gol station in 2001. These clones will also be analysed in two greenhouse trials using the bud puncture method.

The variability of *Ustilago scitaminea,* the causal agent of sugarcane smut, is currently under investigation using 22 simple sequence repeats developed at CIRAD. We intend to study the polymorphism observed between isolates collected around the world. Special attention will be paid to the different Reunion isolates.

Genetic map construction
Sequences differentially expressed in response to challenge by smut (Heinze *et al*., 2001) have been provided by SASEX and are currently being mapped on the studied population. These defense-related genes will be used as RFLP probes. The targeted loci may help us to understand the quantitative variation of smut resistance observed in our trials. RFLP probes linked to the R570 rust resistance gene will also be used. The objective is to determine whether the putative resistance gene identified in MQ 76/53 is carried by a chromosome homologous to the chromosome carrying the R 570 rust resistance gene. Finally, SSR markers will be added to the map in order to anchor the different linkage groups.

Linkage disequilibrium exploitation
Two sets of clones, one highly susceptible to smut and the other highly resistant to smut, will be genotyped using AFLP markers. According to the hypothesis of the existence of a strong linkage disequilibrium among sugarcane cultivar populations (Jannoo *et al*., 1999), interesting associations between markers and smut resistance may be revealed.

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Table 1. Significant marker trait associations at *P*=0.005 (ANOVA).

marker	origin	CGs	HoeneuCUs	Number of whips					BRIX			Diam	Nb flowers		rust		Nb of stalks				
				LP1	LP2	P1	P2	P3	LP1	LP2	P2	LP2	LP1	LP2	P1	P2	LP1	LP2	P1	P2	P3
aagcac_m12	MQ 76/53	8			xxx																
agccaa_m14	MQ 76/53	8			xxx																
aagctg_m11	MQ 76/53	9				xxx															
accaaa_m12	MQ 76/53	11										xxx					xxx				
agcctt_m13	MQ 76/53	11										xxx					xxx				
aagctt_m14	MQ 76/53	11										xxx					xxx				
agcctt_m3	MQ 76/53	11															xxx				
aggcta_m4	MQ 76/53	11										xxx									
agccaa_m8	MQ 76/53	11															xxx				
acccaa_m1	MQ 76/53	12									xxx										
agccta_m12	MQ 76/53	12									xxx										
agccaa_m6	MQ 76/53	12									xxx										
aagcac_m2	MQ 76/53	15									xxx	xxx									
agcctc_m3	MQ 76/53	15										xxx									
aagcac_m5	MQ 76/53	15										xxx									
acactg_m13	MQ 76/53	28													xxx	xxx					
accctt_m10	MQ 76/53	32			xxx																
agccta_m16	MQ 76/53	32		xxx	xxx																
aggcta_m16	MQ 76/53	32		xxx	xxx																
acccac_m2	MQ 76/53	32			xxx																
acctc_m4	MQ 76/53	32			xxx																
acactg_m6	MQ 76/53	32			xxx																
acccttt_m2	MQ 76/53	35																	xxx		
acccttt_m1	MQ 76/53	36											xxx	xxx							
acactg_m7	MQ 76/53	36											xxx	xxx							
aggcta_m6	MQ 76/53	52							xxx	xxx											
actcac_m14	MQ 76/53	63									xxx										
actctt_m17	MQ 76/53	64											xxx	xxx							
acactg_m5	MQ 76/53	64											xxx	xxx							
agccat_m8	MQ 76/53	64											xxx	xxx							
agcctt_m8	MQ 76/53	64											xxx	xxx							
acccaa_m8	MQ 76/53	78											xxx								
actctt_m22	MQ 76/53	79															xxx				
aacctg_m9	MQ 76/53	80												xxx							
accaac_m1	MQ 76/53	81										xxx									
actctt_m13	MQ 76/53	81									xxx										
aggctc_m4	MQ 76/53	89																	xxx		
acgcta_m7	MQ 76/53	89																	xxx		
acgcta_m9	MQ 76/53	89										xxx							xxx		
acgcag_m12	MQ 76/53	94										xxx									
acgcta_m13	MQ 76/53	95																xxx			
acgcta_m10	MQ 76/53	97									xxx										
actcac_m2	MQ 76/53	97									xxx										
acgcag_m14	MQ 76/53	98																		xxx	xxx
aggctc_m9	MQ 76/53	98																		xxx	xxx
acccac_m12	MQ 76/53	115									xxx										
acccaa_m3	MQ 76/53	115							xxx	xxx	xxx										
actctt_m10	MQ 76/53			xxx																	
agccat_m10	MQ 76/53												xxx								
aacctg_m10	MQ 76/53				xxx																
acgcta_m12	MQ 76/53					xxx	xxx	xxx													
aacctg_m13	MQ 76/53													xxx							
acacta_m14	MQ 76/53													xxx							
actcat_m3	MQ 76/53																			xxx	xxx
accctt_m4	MQ 76/53						xxx														
actctg_m5	MQ 76/53										xxx										
agccag_m5	MQ 76/53																		xxx		
acccttt_m7	MQ 76/53								xxx	xxx											
agcctt_m7	MQ 76/53												xxx								
acactc_m7	MQ 76/53			xxx																	
aggctc_m8	MQ 76/53												xxx	xxx							
acccttc_m8	MQ 76/53																			xxx	xxx
acccaa_r13	R 570	22	51		xxx																
acactg_r16	R 570	22	51		xxx																
acactt_r10	R 570	24	9			xxx		xxx													
actcat_r11	R 570	24	9											xxx							
acccttg_r7	R 570	24	9											xxx							
aggctc_r1	R 570	38	29																	xxx	xxx
agcctt_r25	R 570	38	29																	xxx	xxx
acccaa_r14	R 570	39	29		xxx																
acgcag_r15	R 570	39			xxx																
aagctt_rx1	R 570	39			xxx																
aggctc_r12	R 570	70	23					xxx													
acgcag_r6	R 570	74	60													xxx					
agccag_r6	R 570	83	28					xxx													
actcac_r4	R 570	107	22				xxx														
aagctg_r4	R 570	107						xxx													
aggctc_r14	R 570	110	111															xxx			
acgcag_r23	R 570	110	111															xxx			
agccat_r4	R 570		2									xxx									
acactt_r18	R 570		14		xxx			xxx													
aaccac_r34	R 570		52				xxx	xxx													
agcctt_r23	R 570		53									xxx									
aggcta_r10	R 570		54						xxx	xxx											
aagctt_r19	R 570		60												xxx	xxx					
aaccac_r6	R 570		60												xxx	xxx					
actctg_r9R	R 570		60													xxx					
acgctt_r6	R 570		71								xxx										
aagcac_r15	R 570		81												xxx						
aagctt_r3	R 570		93																xxx		
aacctg_r10	R 570														xxx						
acticag_r14	R 570						xxx														
acccac_r21	R 570					xxx															
agccaa_r5	R 570																			xxx	xxx
aacctg_r7	R 570														xxx						
agccag_rx1	R 570			xxx																	
aagctt_rx2	R 570															xxx					
agcctt_rx2	R 570					xxx															
acticag_r4	R 570					xxx															
agccta_rm17	common		4						xxx	xxx	xxx										
acticta_rm8	common		8								xxx										
agcctc_rm25	common		9									xxx									
acccac_rm25	common		15	xxx																	
aaccac_rm14	common		26															xxx			
acccta_rm2	common		33										xxx								
aggctc_rm19	common		49										xxx								
accaaa_rm23	common		52								xxx										
acticag_rm9	common		52								xxx										
agccta_rm26	common		53									xxx									
acticag_rm7	common		54														xxx				
actcac_rm21	common		61								xxx										
acactc_rm17	common		69											xxx							
acactg_rm26	common		103						xxx	xxx											
aggctc_rm2	common		119															xxx			
acticta_rm1	common												xxx	xxx							
acticta_rm11	common											xxx									
acactc_rm16	common											xxx									
agccat_rm19	common																	xxx			
aacctg_rm2	common																		xxx	xxx	xxx
agccta_rm20	common				xxx																
acactg_rm27	common										xxx										
aagctg_rm4	common				xxx																
acccac_rm5	common							xxx													
aagctg_rm5	common						xxx														
acacta_rm9	common						xxx														
agccta_rmJ	common																		xxx	xxx	xxx
agcctc_rmx1	common											xxx									
aggctc_rmx3	common								xxx	xxx											
number of independent QTAs (one QTA per CG)				5	8	6	7	8	7	7	13	12	9	9	5	4	3	5	6	7	7