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Genetic mapping of maize streak virus resistance from the Mascarene source. I. Resistance in line D211 and stability against different virus clones

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Abstract Maize streak virus (MSV) disease may cause significant grain yield reductions in maize in Africa. Réunion island maize germplasm is a proven source of strong resistance. Its genetic control was investigated using 123 RFLP markers in an F_2 population of D211 (resistant) × B73 (susceptible). This population of 165 $F_{2:3}$ families was carefully evaluated in Harare (Zimbabwe) and in Réunion. Artificial infestation was done with viruliferous leafhoppers. Each plant was rated weekly six times after infestation on a 1–9 scale previously adjusted by image analysis. QTL analyses were conducted for each scoring date, and for the areas under the disease, incidence and severity progress curves. The composite interval mapping method used allowed the estimation of the additive and dominance effects and QTL × environment interactions. Heritabilities ranged from 73% to 98%, increasing with time after infestation. Resistance to streak virus in D211 was provided by one region on chromosome 1, with a major effect, and four other regions on chromosomes 2, 3 (two regions) and 10, with moderate or minor effects. Overall, they explained 48–62% of the phenotypic variation for the different variables. On chromosome 3, one of the two regions seemed to be more involved in early resistance, whereas the second was detected at the latest scoring date. Other QTLs were found to be stable over time and across environments. Mild QTL × environment interactions were detected. Global gene action appeared to be partially dominant, in favor of resistance, except at the earliest scoring dates, where it was additive. From this population, 32 families were chosen, representing the whole range of susceptibility to MSV. They were tested in Réunion against three MSV clones, along with a co-inoculation of two of them. Virulence differences between clones were significant. There were genotype × clone interactions, and these were more marked for disease incidence than for severity. Although these interactions were not significant for the mean disease scores, it is suggested that breeders should select for completely resistant genotypes.

Key words Quantitative trait loci · Disease resistance · MSV · Composite interval mapping · Tropical maize

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Introduction

Streak is an important endemic disease in Africa (Thottappilly et al. 1993) caused by the maize streak virus (MSV), a geminivirus transmitted by insect vectors of the *Cicadulina* genus. MSV occurs throughout sub-Saharan Africa and in the Mascarene islands (Indian Ocean), from sea level up to 2500 m elevation. Inter- and intra-isolate molecular diversity have been reported (Briddon et al. 1994; Isnard et al. 1997; Ngwira et al. 1997), and isolates with different aggressiveness have been identified (Rodier 1995). Maize is one of the favourite hosts of this virus (Rose 1978). Some weeds are likely involved in its perpetuation (Mesfin et al. 1992).

Initial symptoms are round yellow spots scattered on the youngest leaves. Subsequent leaf tissues exhibit chlorotic streaks at an increasing density. When the infection occurs on young susceptible seedlings, the chlorosis ultimately covers the entire area of the leaf, stalk

internodes are reduced, and ears are poorly filled or do not develop at all. There may be necrosis and death of the plant. Very substantial damage, including total yield loss, has been observed in maize crops. During the 1980s, several epidemics were reported in African countries (Rossel and Thottappilly 1985; Malithano et al. 1987; Kim et al. 1989). However, these outbreaks have been erratic and very difficult to predict.

Development of MSV-resistant maize varieties is an efficient way to reduce the impact of this disease. Several sources of MSV resistance have been identified in South Africa (Storey and Howland 1967), Nigeria (Soto et al. 1982; Efron et al. 1989), Burundi (Zeigler and Manirakiza 1986), and the Mascarenes (Etienne and Rat 1973). Genetic resistance in the South African source was reported to be simply inherited (Storey and Howland 1967), whereas it appeared to be oligogenic in IB32, a line developed from the Nigerian resistance source (Kim et al. 1989). In Réunion, the IRAT297 composite was created by intermating 41 Mascarene populations (including cv 'Revolution') and a South African line (Hainzelin and Marchand 1986). Inbreds presenting complete resistance were selected from an improved form (CVR3-C3) of this population. According to a quantitative genetic study (Rodier 1995), the complete resistance of one of these inbred lines (D211) seems to be oligogenic. Moreover, several genetic factors controlling partial resistance seem to be present.

New methods that enable the genetic dissection of complex traits (quantitative trait locus or QTL mapping) have been developed in recent years, as the number of available molecular markers have increased, thus permitting good genome coverage. The simplest method described by Soller et al. (1976) allowed detection of marker-QTL associations through variance analysis. The simple interval mapping (SIM) method, implemented by Lander and Botstein (1989), improved the efficiency by testing for the presence of a QTL in each marker-interval on a whole genetic map. Recently, Jansen (1993) and Zeng (1994) proposed to increase the precision of mapping multiple QTLs by using markers as cofactors in a mixed model combining simple interval mapping with multiple regression. This latter composite interval mapping (CIM) method was completed by Jiang and Zeng (1995) for the purposes of performing multiple trait analyses. Genetic effects such as additivity, dominance, along with pleiotropy and QTL × environment interactions can be tested and estimated.

As the resistance originating from Réunion was extremely strong, apparently involving one major and one minor genetic system (Rodier et al. 1995), genetic mapping was undertaken in the D211 resistant line using the composite interval mapping method (Jiang and Zeng 1995). Some families used for this mapping experiment were also tested against MSV clones of different pathogenicity in order to examine the resistance stability. The objectives of the investigation presented here were then: (1) to identify QTLs responsible for resistance to MSV at different dates, and also for two components of the disease, estimate their genetic effects, and look for QTL × environment interactions, (2) to determine whether there were any genotype × clone interactions, and discuss the possible consequences.

Material and methods

Plant material and experimental design

An F_2 population segregating for MSV resistance was developed. The resistant parent was D211, a line (S_5 generation) selected in Réunion island for complete resistance to MSV (Rodier et al. 1995). It was extracted from a tropical composite population (CVR3-C3) resistant to the streak, mosaic and stripe viruses and specifically improved for streak resistance. The susceptible male parent was B73, an American inbred line from the Stiff-Stalk group.

A single F_1 partially resistant plant from the D211×B73 cross developed at CIRAD in Réunion island in 1993, was selfed. Selfing of each F_2 plant was done during the Southern hemisphere winter of 1994, when MSV pressure was quite low. Each of these $F_{2:3}$ families was then multiplied by sib-mating 21 plants at the CIMMYT station in Tlaltizapán, Mexico during the summer of 1995. A total of 165 families (population D211×B73, hereafter called DB) yielded sufficient seed quantities for the trials.

Trials were carried out in two different locations. In Harare (CIMMYT station, Zimbabwe), local resistant checks, Kilima S4–8 and Kilima S4–12, and a susceptible check CG4141 were used. In Saint Pierre (CIRAD station, Réunion), the CIRAD390 line (Clerget et al. 1996) and the Sabrina hybrid from Pioneer-France Maïs were used as resistant and susceptible checks, respectively. The 180 entries (*i.e.* 165 F_3 families, both parents, and seven susceptible and six resistant checks) were planted in a 15×12 row-column design (John and Eccleston 1986) with two replications on Nov. 20, 1995 in Harare and March 19, 1996 in Réunion. A row-column design presented the advantage that it ensured a good control of both the soil variability, whatever the fertility gradient, and the infestation process variability between rows (see below). Plots consisted of a single row of 21 plants, 4 m long. Rows were separated by 0.75 m. A complete "row" in the design thus consisted of a series of twelve 4-m plots, plus pathways. Plots were oversown, by hand, with two or six seeds per hill, depending on the location. Plants were randomly thinned to 7 plants per square meter before infestation in Réunion, and after infestation in Harare.

Resistance evaluation

Infestation and disease assessment

Resistance to maize streak virus needs to be evaluated by artificial infestation in order to control the whole procedure. Since no mechanical inoculation is possible, each plant was artificially infested with about three mass-reared viruliferous anaesthesized insect vectors, *Cicadulina mbila* (leafhopper), when the plantlets had three to four fully expanded leaves in Harare (Dec. 7, 1995) and two to three in Réunion (March 29, 1996). The infestation process was generally conducted by one person along each row of the design. At CIRAD Réunion, the *C. mbila* population is the result of selection for a 100% MSV transmission rate (Reynaud 1988). The infestation isolate used in the selection program, called isolate M, is maintained in the field on partially resistant plants by keeping some of these plants after each screening trial as source plants for the next infestation. This isolate is thus a viral population that is permanently exposed to resistant varieties (Rodier 1995). At CIMMYT Harare, isolates were collected once at the beginning of the 1980s in the field on susceptible plants. Since then, stocks of streak-diseased susceptible plants have been maintained in a confined environment. The isolate used for the trials was therefore not

subjected to selection pressure present at the station. Moreover, genetic drift may have reduced its initial variability.

Symptoms were evaluated on the last fully expanded leaf of each plant on a 1–9 scale [from resistant (no symptoms at all) to susceptible (leaf fully chlorosed, plant almost dead)] once per week until 42 days after infestation (dai). Each grade of this scale is proportional to the chlorotic area of the leaf, as previously determined by image analysis (Rodier 1995). This scale is also correlated to the virus concentration in the leaf and to the chlorophyll concentration (Rodier 1995).

Variable description

These ratings allowed us to quantify the overall resistance and two of its components. The variable MSV_u is the disease score given to each individual plant, at the u^{th} dai. The variable PIS_u, related to the incidence of the disease, is the proportion of plants without any symptom in each plot, at the u^{th} dai. The severity of the disease, NMS_u, was calculated as the mean score of all plants showing symptoms in each plot, at the u^{th} dai. When no plant presented symptoms in a plot, NMS_u was given the value 0. In order to integrate all of these variables over time, the area under the disease progress curve (AUDPC) was calculated – called AUT for the disease score, APIT for incidence, ANMT for severity – according to the general formula described in Ceballos et al. (1991). The first considered interval of time was between 0 and 7 dai. At 0 dai, all plants were healthy. Total length of time of the disease study was 42 dai in Harare and 35 dai in Réunion.

Restriction fragment length polymorphism (RFLP) genotyping

Leaf samples from each F_2 plant were harvested in September–October 1994, quick-frozen in liquid nitrogen, and lyophilized in Réunion. They were then packed in air-proof plastic bags, sent quickly to Montpellier (France), and stored at –20°C before being ground and put into small plastic vials that were also stored at –20°C. RFLP analysis was performed at CIMMYT (Mexico). DNA was extracted [modified from Saghai-Maroof et al. (1984) in Hoisington et al. (1994)] purified, quantified, and quality-controlled before being digested with one of two restriction enzymes (*Eco*RI or *Hind*III). Fragments were then separated by electrophoresis in 0.7% agarose double gels and then transferred onto non-charged nylon membranes by Southern blotting. Probes were labeled with digoxygenine and hybridized onto the membranes. Polymorphism was revealed by the reaction between the antibody antidigoxygenin-alkaline phosphatase and its substrate, AMPPD, or CSPD (chemiluminescence detection). For more details see Hoisington et al. (1994).

A selection of probes from different origins (maize genomic DNA: agr, Agrigenetics; asg, Asgrow Seed; bnl, Brookhaven National Laboratories; npi, Native Plants Incorporated; umc, University of Missouri-Columbia; maize cDNA: csu, California State University; bcd and cdo Cornell University, respectively barley and oat leaf cDNAs) were screened and hybridized onto the whole population, when polymorphic. Genetic data were captured and verified by two different readers, using HyperMapData software (Hoisington et al. 1993).

Data analyses

Map construction

Possible segregation distortion, compared to the expected Mendelian proportions, was determined at each marker locus using both a chi-square test and the sequentially rejective Bonferroni procedure (Holm 1979). The genetic map, based on 172 F_2 individuals, was constructed using the software MAPMAKER 2.0 (Lander et al. 1987). Linkage between two markers was declared significant when the Lod (Log_{10} of the likelihood odds ratio) score exceeded 3.0 and the recombination frequency was below 0.4. Markers were ordered by multipoint analyses. Recombination frequencies were transformed into genetic distances using the Kosambi mapping function (Kosambi 1944).

Field data

In order to obtain good predictors of the genotypic value of each family, analyses of variance were conducted within each environment on a plot-mean basis or on an individual basis, depending on the variable, using the SAS Mixed procedure (SAS 1997). All factors were considered to be random. On a plot-mean basis, total variation was partitioned into effects of replications, rows, columns, family genotypes, and errors:

$$Y_{ijkl} = \mu + Rep_i + (Row/Rep)_{ij} + (Col/Rep)_{ik} + G_l + e_{ijkl}$$

where Y_{ijkl} is the variable measured on the l^{th} $F_{2:3}$ family genotype of the i^{th} rep, at the intersection of the j^{th} row and the k^{th} column, and μ is the general mean of the trial. On an individual basis, we broke down the residual variation into the effects of the plot and of the non-controlled micro-environment plus the genetic effect at the plant level. The plot effect was also confounded with the interaction between families and replications. Normality of residual distributions was tested using the SAS Univariate procedure (SAS 1997). BLUPs (best linear unbiased predictors, Henderson 1975) of each family were obtained by adding the general mean of the trial to the solution of the random "genotype" effect. Broad-sense heritability ($\hat{h}^2_{SL}$) at the experimental design level was estimated overall for both environments, with the location effect considered as fixed and genotype × environment (G×E) interactions as random:

$$\hat{h}^2_{SL} = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_G + \frac{\hat{\sigma}^2_{G\times E}}{p} + \frac{\hat{\sigma}^2_e}{rp}}$$

and

$$\hat{h}^2_{SL} = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_G + \frac{\hat{\sigma}^2_{G\times E}}{p} + \frac{\hat{\sigma}^2_{plot}}{rp} + \frac{\hat{\sigma}^2_{WF}}{rpn}},$$

n being the number of plants per plot (*n*=21), r the number of replications (r=2), and p the number of locations (p=2), respectively, for variables on plot and on individual bases; $\hat{\sigma}^2_G$, $\hat{\sigma}^2_{G\times E}$, $\hat{\sigma}^2_e$, $\hat{\sigma}^2_{plot}$, $\hat{\sigma}^2_{WF}$ are the variance estimates of the corresponding effects (WF, within family).

Quantitative trait locus (QTL) analyses

Method for QTL detection

QTL detection was performed using the multiple trait analysis described in Jiang and Zeng (1995) with the corresponding computer program. BLUPs of each family in each environment for each trait were used in the analysis.[1] At first, simple interval mapping (SIM) (Lander and Bostein 1989) was performed, which corresponds to model III of Zeng (1994) in his notation, and markers were chosen at the peaks where QTLs were suggested. Secondly, the selected markers were used as cofactors in the analysis when they were unlinked with the region being screened in order to reduce the residual variation and increase the QTL detecting power (model II). Finally, flanking markers of the testing interval at least 20 cM away were also included in the model as cofactors for separating possible linked QTLs (model I). In the case of joint analysis of one trait

[1] Nonetheless, one family of the DB population had missing field data in the Réunion trial. As suggested by Jansen and Stam (1994), this family, which was bringing valuable genotypic data and field data in one environment, was kept. As Jiang and Zeng's program did not accept missing phenotypic data, its value was evaluated by comparison with other families showing the same genotypic data at QTLs for the AUT variable in Réunion and Zimbabwe

for the two environments at the same time or several traits in the same environment, cofactors were selected based on the joint analysis or combined from separate analyses.

Determination of thresholds

The threshold for declaring a QTL present was chosen in two different ways. The first type of threshold was considered with respect to marker-assisted selection. In this case, in the separate analysis for each trait in each environment, a QTL was declared significant: (1) when a peak in the LOD profile presented a value above 3.0 under model I, or (2) when a peak was persistent under model I and II and the LOD could be above 3.0 only for model II. Based on the discussion of Lander and Bostein (1989), a LOD of 3.0 would be quite a conservative threshold in a population of maize with ten chromosomes with marker intervals of 10 cM by SIM. Simulations carried out by Goffinet and Mangin (1998) showed that the threshold used in SIM is conservative for composite interval mapping (except for two closely linked QTLs). The second type of threshold was chosen very loosely in order to reflect the consistency over the two environments for some QTLs and to compare putative QTLs with those of other populations. Namely, some QTLs can be significant in one population but not in another, or non-significant at LOD 3.0 in two populations but at the same location in the genome. A QTL was thus declared to be putative when the LOD was between 2.0 and 3.0 with model I and not above 3.0 with model II.

In the joint analysis of more than one trait or environment, the threshold has to be higher since more tests are performed. Determination of the threshold in such a case was discussed in Jiang and Zeng (1995). With a threshold of LOD 3.0 in a single-trait analysis, which corresponds to a χ^2 value of 13.8 (LR threshold), the type-I error rate is 0.0033 for three degrees of freedom in an F_2 population (for additive and dominant effects and for the position of the QTL in the testing interval). With the same type-I error rate for m traits, the degree of freedom would be equal to 2m+1. Then, the LR threshold for the joint analysis of two traits would be equal to $\chi^2_{0.0032;5}$=17.8. When a QTL was detected in the joint analysis, QTL × environment interactions were also tested. The LR threshold for this test was set at 5.99 (Jiang and Zeng 1995). All results are given by LR values, which is the standard output of the program. The LR value is equal to 2 ln10, the value of the LOD score, and LR values of 17.8, 13.8, 11.5, 9.2 correspond, respectively, to LODs of 3.9, 3.0, 2.5, 2.0.

The QTL position was estimated at the peak of the LR curve. Additive and dominant effects (*a* and *d*, respectively) were estimated under model I for each QTL in order to reduce noise from nearby regions. The type of gene action in the F_2 generation was determined on the basis of the dominance ratio (DR=|2*d*/*a*|) by the same criteria as used by Stuber et al. (1987): additive for DR<0.2, partial dominant for 0.2=<DR<0.8, dominant for 0.8=<DR<1.2, and overdominant for DR=>1.2. *d* had to be multiplied by 2 as it was estimated from F_3 families.

Proportion of the explained variation

Estimation of the genotypic variance among F_3 lines contributed by the ith QTL was calculated as: $\hat{a}_i^2/2+\hat{d}_i^2/4$, (Falconer 1989), where $\hat{a}_i$ and $\hat{d}_i$ are the additivity and dominance estimates, respectively, for this QTL. The proportion of phenotypic variation explained by the ith QTL was then $R_i^2=(\hat{a}_i^2/2+\hat{d}_i^2/4)/\hat{\sigma}_P^2$, with $\hat{\sigma}_P^2$, equal to the total variation among BLUPs of the trait. The total percentage of the phenotypic variation explained by all the significant QTLs identified for one trait (R^2) was calculated by multiple regression with the nearest marker(s) of each QTL as factors. Total additive and dominance effects were estimated by summing all individual effects at the identified QTLs.

Virus clone × maize genotype interactions

Choice of actors

In order to study virus clone by maize genotype interactions, we identified a range of families, representing different levels of resistance and with different sets of the most important QTLs, and a range of clones, representing different levels of pathogenicity, with different mutations. Thirty-two families were chosen among the 165 available. Three clones (R4, R2, RX) were chosen among those extracted by Isnard (1998) from the N2A isolate which is characterized by high pathogenicity (Rodier 1995). R4 and R2, compared to a consensus MSV sequence, presented mutations in distinct regions[2], probably intervening in the expression and function of their viral proteins (Isnard 1998). RX was not sequenced. The pathogenicity of these three clones was assessed in a range of maize lines and one hybrid, all differing in their levels of resistance. R4 showed the mildest pathogenicity, R2 an intermediate pathogenicity, and RX an intermediate to high pathogenicity, depending on the trials (Isnard 1998). We conducted four experiments, three with each of the different clones and one with a mixture of R2 and R4, to test for complementation that could result in higher pathogenicity.

Resistance evaluation

Susceptible maize plantlets were infected by *Agrobacterium* at CIRAD (France) with one of these three clones or the mixture, then sent to Réunion for virus acquisition by leafhoppers. Family plantlets (2- to 3-leaf stage), primarily grown in an insect-proof environment, were infested by the leafhoppers in insect-proof cages for 48 h. Each cage included 16 families of 24 plants each, the susceptible check (B73) and one of the two resistant checks (D211 or CIRAD390). Plantlets were transplanted under insect-proof tunnels in December 1996.

For each experiment, the 32 families were evaluated in an alpha-lattice design. Each alpha-lattice was composed of two replications of two incomplete blocks, each comprising 18 plots, 16 families and two checks. Each incomplete block corresponded to one insect-proof tunnel and one infestation cage. First replications of each experiment were handled together, followed by the second replications.

Symptoms were evaluated for 4 weeks according to the procedure described previously. For each family, mean score, incidence, and severity were analyzed for the four scoring dates.

Data analysis

Each alpha-lattice was analysed separately. Then a global model, allowing detection of clone × genotype interactions, was used:

$$Y_{ijklm}=\mu + Clone_i + (Rep/Clone)_{ij} + (Block/(Rep \times Clone))_{ijk} + G_l + (Clone \times G)_{il} + e_{ijklm}$$

with Y_{ijklm} being the variable measured on the mth plant of the lth $F_{2:3}$ family genotype in the kth block of the the jth rep in the experiment conducted with the ith clone, and μ being the general mean of the trial. All effects other than interactions were considered as fixed. Clone and family means were compared with the Newman-Keuls test. The SAS GLM procedure (SAS 1997) was used for all calculations.

[2] The EMBL accession numbers of the nucleotide sequences of R2 and R4 are AJ224504 and AJ224506, respectively.

Results

RFLP linkage map

Of the screened probes 84% detected polymorphism between the parental lines. Many of them allowed us to map multiple loci. However, we discarded some loci which formed a cluster with other markers so that the power of QTL detection would not be diminished. We mostly selected codominant markers that were spread evenly over the genome. Using the Bonferroni procedure (Holm 1979), none of the selected markers showed significant segregation distortion. Nevertheless, when individual tests were performed, 4 markers of the same region on chromosome 2 exhibited some distortion in favor of the B73 parent, with a type-I error of α=0.01 for 3 of them, and α=0.05 for the other 1.

The final map (Fig. 1) was constructed with 123 RFLP (111 codominant and 12 dominant) markers. Ten linkage groups were obtained, totaling 1454 cM. The *umc53a* distal marker was attributed to chromosome 2, on the basis of the 1995 UMC reference map (Coe et al. 1995), although it was linked only at Lod 2 to the closest marker of this chromosome. The order of the markers was in good agreement with the reference map, which allowed identification of bins (portions of each chromosome), as defined in Coe et al. (1995). The average distance between 2 markers was 12.8 cM with a standard deviation of 8.0 cM. The good coverage of the genome map allowed an extensive search for QTLs.

Field trait analyses

The artificial inoculations were successful: the susceptible checks exhibited heavy symptoms and the susceptible parent B73 was almost completely dead in all trials, at the latest 28 days after infestation. The resistant parent D211 did not show any symptoms. Other checks from Zimbabwe were partially resistant (Fig. 2).

Genotypic variance components, which were highly significant for all traits at both locations, were larger in Réunion than in Harare (Table 1). Normality of residuals distributions, tested *a posteriori*, was met for the variables PIS14, NMS35, NMS42, APIT in Harare and for PIS07 in Réunion. The hypothesis of normality was rejected for the other variables due to a high Kurtosis value. All distributions, except MSV07 and NMS07 in Harare, were symmetrical. If symmetrical, results of analysis of variance are less affected by the non-normality. Heritabilities ranged from 73% for MSV07 to 98% for AUT, PIS14, and APIT, increasing with time after infestation (data shown for AUT, APIT and ANMT in Table 1). This indicates that relative genotypic variation was greater as the epidemic progressed. These very high heritabilities demonstrated a tremendous genotypic variability between the F_3 families as compared to the environmental variability. The locations were significantly different for all scoring and severity variables except NMS35. Differences were greater for the earliest scoring dates. F_3 family means were always higher in Réunion than in Harare. There was no difference between the two locations for variables related to disease incidence, except for PIS07. The genotype × environment interaction variance component ($\hat{\sigma}^2_{G\times E}$), was significant for all disease scores (MSV_u and AUT). For the variables related to disease severity (NMS_u and ANMT), $\hat{\sigma}^2_{G\times E}$ was significant at all scoring dates but 7 dai, whereas for the variables related to disease incidence (PIS_u and APIT), $\hat{\sigma}^2_{G\times E}$ was significant only at 7 dai. Some biological conditions specific to each location may have influenced disease severity differently, depending on the family genotype. However, the magnitude of these significant interactions was low relative to variations among families, except for the earliest disease scores.

Resistance to MSV is a quantitatively inherited character, as shown by the distribution of resistance values (BLUPs) obtained per family within each environment (Fig. 3). In all cases, F_3 family means were lower than the mid-parental value, indicating partial dominance of resistance. As the parental lines represented the boundaries of the distribution at all scoring dates, no transgression could be detected among F_3 families. Moreover, the distribution of individual scores (summarized by AUT) suggests the presence of two systems, one major conferring strong resistance and the other, polygenic, conferring partial resistance (Fig. 3).

QTL analyses

QTLs were identified on several chromosomes (chr), explaining both a large proportion of the phenotypic variation and a very small one, which supports the above hypothesis of major and minor genetic systems (Fig. 1). At least 5 significant QTLs were detected with an LR above 13.8 in bins 1.05, 2.03, 3.02/03, 3.09 and 10.05 (Table 2). Five more putative QTLs (LR between 9.2 and 13.8) were detected in bins 1.09, 2.05, 2.09, 8.06 and 9.01. Some regions of the genome were also detected only by joint analysis, when taking into account both environments at the same time, on chromosomes 2 and 8 (data not shown). On chromosome 2, joint analysis allowed the detection of 1 QTL, at 64 cM for MSV07, 90 cM for MSV21, 90 cM for MSV28, 77 cM for MSV35 (at this date in Réunion, a significant QTL was detected at 49 cM and a putative one at 86 cM), and at 90 cM for APIT. On chromosome 8, another QTL was detected by joint analysis for the AUT variable at 58 cM (bin 8.03).

The 2 QTLs in bins 1.05 and 10.05 were stable for all dates and environments (Table 2). The significant QTL on chromosome 2 (bin 2.03) was detected for MSV35 and ANMT in Réunion. On chromosome 3, the action of QTLs appeared to vary with time after infestation. The QTL in bin 3.09 was detected at 7 dai at both locations. At 14 dai, it was still significant in Réunion but had became putative in Harare. For later scoring dates (MSV21, MSV28, MSV35) and for AUT, APIT and

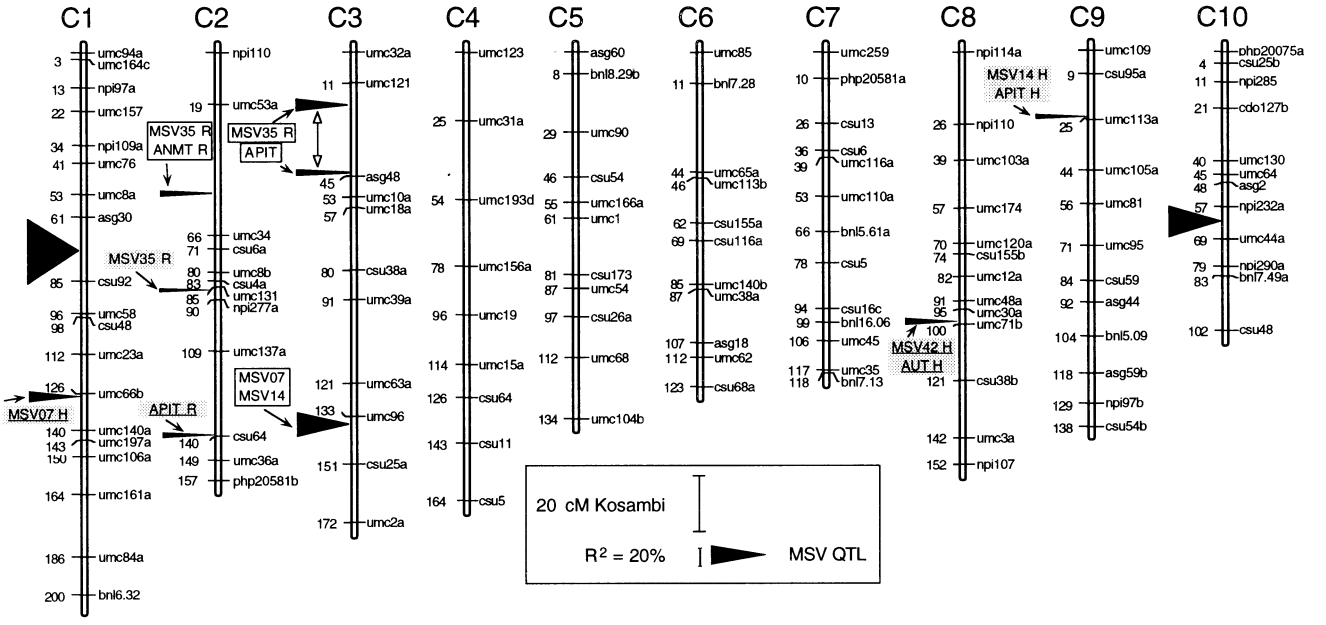

Fig. 1 RFLP linkage map and location of QTLs for MSV resistance detected in 165 $F_{2:3}$ families of the cross D211 (MSV resistant)×B73 (MSV susceptible). Locus names are on the *right* and cumulative distances in centi Morgans are on the *left* of each maize chromosome designated by the letter *C*. *Tops* of *triangles* mark LR peak positions of each QTL. The *width* of the *triangle basis* is proportional to the percentage of the phenotypic variation (R^2) explained by that QTL. If the QTL was detected for specific variables, the corresponding variable names (described in the Materials and methods) followed by the location designation are indicated in *boxes beside* the *triangles*. The R^2 mean for these variables is represented. The Harare (Zimbabwe) location is designated by an H; the Réunion island location is designated by an R. No location designation indicates that the QTL was detected with a LOD above 3 at least in one environment, and with a LOD at least above 2 in the second environment. An *underlined* variable name indicates that the allele increasing MSV resistance may have been contributed by the susceptible parent. A *white box* indicates a significant QTL detected with a LOD above 3. In order to know if the QTL was detected putatively for other variables, refer to the text. A *light grey color* indicates a putative QTL detected with a LOD between 2 and 3. *No box* indicates a significant QTL detected for all variables at both locations. In this case, the QTL was represented for the AUT variable, with the R^2 averaged across the two environments. Two QTLs linked by a *hollow arrow* are considered to be in the same region of the genome

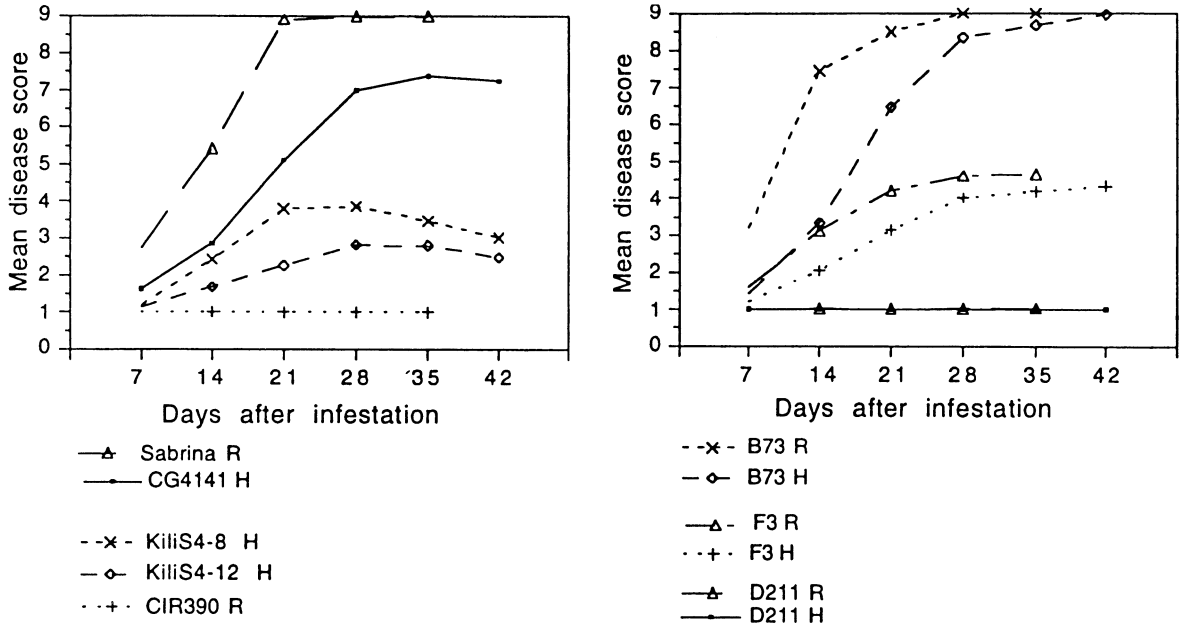

Fig. 2 Time-course of the disease symptoms on the susceptible checks (CGR4141 in Harare, Sabrina in Réunion), on the resistant checks (Kilima S4–12 and Kilima S4–8 in Harare, CIRAD390 in Réunion), on the susceptible parent B73, on the resistant parent D211 and on the $F_{2:3}$ families in Harare (*H*) and Réunion (*R*)

Table 1 Means of parents, checks, and 165 F_3 families; significance of the fixed effect location and estimates of the variance components and heritabilities among F_3 lines for area under the disease progress curve over 42 days (AUT), integration over time of the proportion of symptom-free plants per plot (APIT), integration over time of the mean scoring of plants presenting symptoms per plot (ANMT)

	Genotype[a]		AUT			APIT			ANMT		
			Harare	Réunion	H and R [b]	Harare	Réunion	H and R	Harare	Réunion	H and R
Means[a]	P1	D211	1.00±0.00	1.00±0.03		91.75±0.00	89.82±0.28		0.08±0.00	0.20±0.10	
	P2	B73	5.54±0.07	6.63±0.04		9.62±2.13	0.10±0.00		5.63±0.14	6.63±0.09	
	SC	CG4141	4.69±0.03			6.63±0.93			4.75±0.06		
		Sabrina		6.23±0.02			0.27±0.09			6.22±0.04	
	RC	KiliS4–12	2.07±0.08			39.19±1.74			2.70±0.04		
		KiliS4–8	2.79±0.03			15.68±0.91			2.95±0.03		
		CIR390		1.00±0.00			89.79±0.17			0.23±0.07	
	F_3		2.87±0.02	3.26±0.02	3.06±0.01	35.16±1.30	30.15±1.45	32.66±0.98	3.30±0.05	3.70±0.06	3.50±0.04
Fixed effect	Location[d]				*			NS			*
Variances[e]	$\hat{\sigma}^2_G$	F_3	1.09±0.12	1.66±0.19	1.34±0.15	510.88±58.99	638.81±72.80	575.32±65.01	0.80±0.09	1.15±0.13	0.97±0.11
	$\hat{\sigma}^2_{G\times E}$		–		0.04±0.01	–	–	5.02±3.23	–	–	0.02±0.01
	$\hat{\sigma}^2_{plot}$		0.01±0.01	0.04±0.01	0.02±0.01	–	–	–	–	–	–
	$\hat{\sigma}^2_{WF}$ or $\hat{\sigma}^2_e$		0.77±0.01	0.97±0.02	0.87±0.01	38.85±4.66	33.67±4.16	37.37±3.16	0.07±0.01	0.11±0.01	0.09±0.01
$\hat{h}^2_{SL}$ design[f]			0.98	0.98	0.97	0.96	0.97	0.98	0.96	0.95	0.96

All variances $\hat{\sigma}^2_G$ were significant at 1‰. All variances $\hat{\sigma}^2_{G\times E}$ were significant at 1‰ except for APIT where it was non-significant. The variance $\hat{\sigma}^2_{plot}$ for AUT was non-significant in Harare, significant at 1% in Réunion, significant at 1 ‰ for combined analysis of both environments

[a] P1, P2, Parent 1 and 2, respectively; SC, RC susceptible and resistant checks, respectively; CIR: CIRAD; Kili: Kilima

[b] H, Harare, R, Réunion Island

[c] ±Standard errors

[d] Significant at the 0.05 probability level; NS, non-significant

[e] $\hat{\sigma}^2_G$, $\hat{\sigma}^2_{G\times E}$, $\hat{\sigma}^2_{plot}$, $\hat{\sigma}^2_{WF}$ Estimates of the variances between families, of families×environment interactions, between plots, and within family, respectively

[f] $\hat{h}^2_{SL}$, Broad-sense heritability at the experimental design level

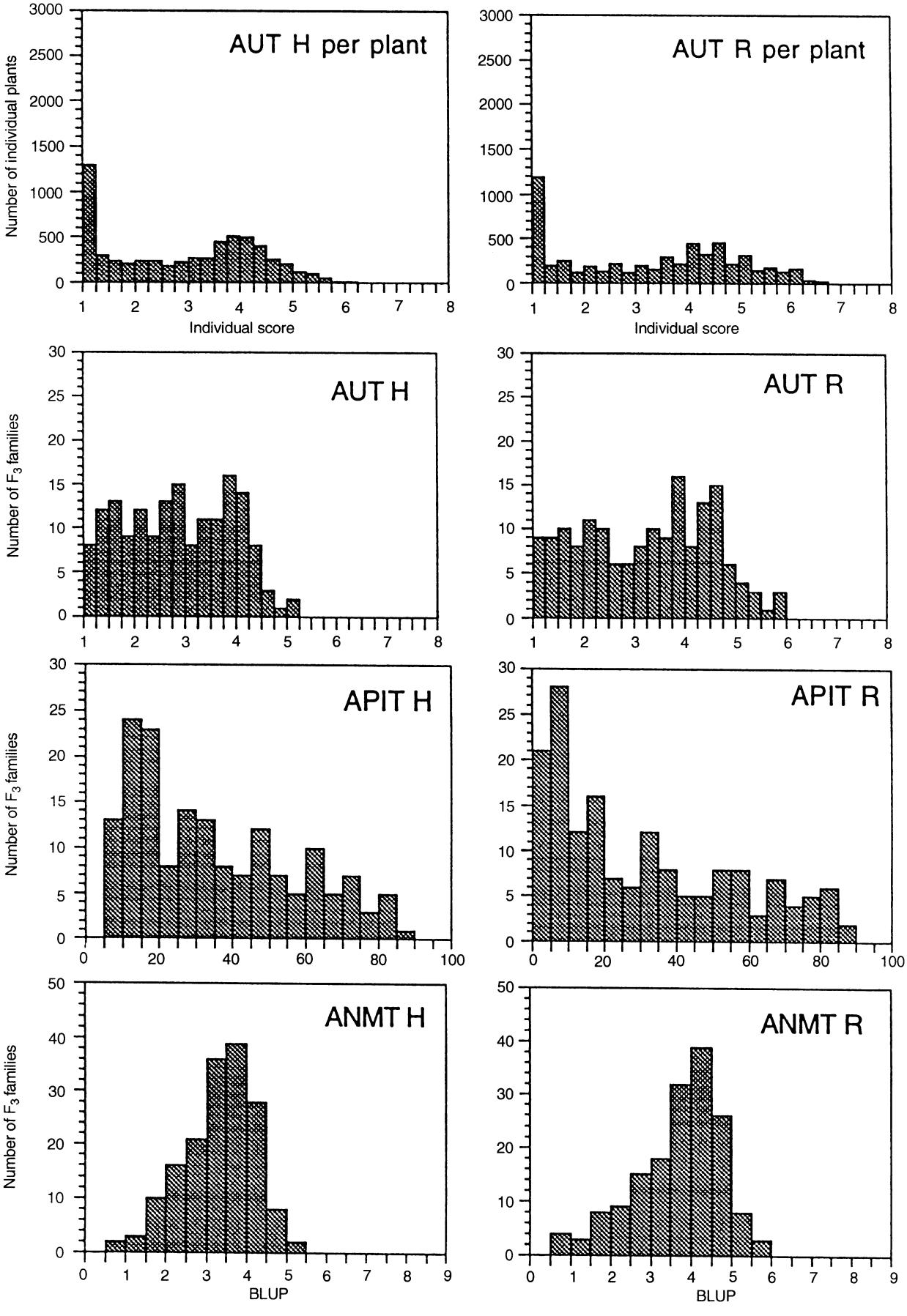
AUT H per plant
AUT R per plant
AUT H
AUT R
APIT H
APIT R
ANMT H
ANMT R
Number of individual plants
Number of F_3 families
Individual score
BLUP

Table 2 Genetic characteristics of significant QTLs in two different environments for scoring variables at different dates i (MSVi); for integration over time of these scoring variables (AUT), of the proportion of symptom-free plants per plot (APIT), and of the mean score of plants presenting symptoms per plot (ANMT). Terms in italics indicate that the QTL is detected at a non-significant level

Variable	Place[a]	Chr	Bin and marker interval[b]	Centi Morgans[c]	LR[d]	*a*[e]	*d*[e]	R[f]	Action[g]
MSV07	H	1	1.05	71	53.3	–0.13	–0.02	46.9	PD
	R	1	asg30 (61 cM)	71	81.9	–0.45	–0.07	58.8	PD
	QTL×E	1	csu92 (85 cM)	70	76.0				
	H	3	3.09	133	18.2	–0.04	0.07	29.8	OD
	R	3	umc96 (133 cM)	137	14.2	–0.12	0.19	24.4	OD
	QTL×E	3	csu25a (151 cM)	137	9.2				
	H	10	10.05	64	31.0	–0.09	–0.04	26.1	D
	R	10	npi232a (57 cM)	60	23.0	–0.17	–0.15	20.5	OD
	QTL×E	10	umc44a (69 cM)	59	16.8				
MSV14	H	1	1.05	71	100.9	–0.73	–0.11	72.2	PD
	R	1	asg30 (61 cM)	72	111.3	–1.62	–0.10	71.7	A
	QTL×E	1	csu92 (85 cM)	72	101.0				
	H	*3*	*3.09*	*136*	*12.5*	*–0.16*	*0.27*	*21.9*	*OD*
	R	3	umc96 (133 cM)	135	13.9	–0.35	0.55	19.7	OD
	QTL×E	*3*	*csu25a (151 cM)*	*135*	*10.6*				
	H	10	10.05	62	27.6	–0.23	–0.30	29.3	OD
	R	10	npi232a (57 cM)	61	29.7	–0.48	–0.67	30.5	OD
	QTL×E	10	umc44a (69 cM)	58	25.3				
MSV21	H	1	1.05	72	99.1	–1.39	–0.07	64.6	A
	R	1	asg30 (61 cM)	71	97.1	–2.00	0.09	62.1	A
	QTL×E	1	csu92 (85 cM)	72	61.0				
	H	10	10.05	62	29.2	–0.38	–0.60	28.9	OD
	R	10	npi232a (57 cM)	62	37.3	–0.71	–0.92	33.9	OD
	QTL×E	10	umc44a (69 cM)	62	30.6				
MSV28	H	1	1.05	73	112.4	–2.00	–0.17	69.3	A
	R	1	asg30 (61 cM)	72	104.5	–2.05	0.10	61.4	A
	QTL×E	1	csu92 (85 cM)	72	9.9				
	H	10	10.05	62	27.4	–0.50	–0.80	26.0	OD
	R	10	npi232a (57 cM)	64	38.3	–0.75	–0.93	33.1	OD
	QTL×E	10	umc44a (69 cM)	66	16.3				
MSV35	H	1	1.05	72	117.0	–1.92	–0.17	69.5	A
	R	1	asg30 (61 cM)	72	96.9	–2.00	0.03	56.5	A
	QTL×E	1	csu92 (85 cM)	72	6.3				
	H	*2*	*2.03*	*49*	*5.5*	*–0.40*	*–0.04*	*3.0*	*A*
	R	2	umc53a (19 cM)	49	10.8	–0.64	–0.06	5.8	A
	QTL×E	*2*	*umc34 (71 cM)*	*49*	*8.6*				
	H	*3*	*3.02*	*25*	*9.0*	*–0.39*	*–0.41*	*9.2*	*OD*
	R	3	umc121 (11 cM)	18	13.8	–0.52	–0.56	12.4	OD
	QTL×E	*3*	*asg48 (45 cM)*	*18*	*7.4*				
	H	10	10.05	63	31.1	–0.56	–0.82	30.4	OD
	R	10	npi232a (57 cM)	64	39.3	–0.82	–0.94	34.2	OD
	QTL×E	10	umc44a (69 cM)	64	15.1				
MSV42	H	1	1.05	73	106.7	–1.89	–0.13	65.5	A
	H	10	10.05	65	30.2	–0.66	–0.77	29.5	OD

◀ **Fig. 3** Distributions of individual integrations over time of scorings (AUT per plant) and of BLUPs obtained per family for AUT, APIT (disease incidence integrated over time) and ANMT (disease severity integrated over time) in 165 segregating F_3 families from the D211×B73 cross in two different environments: Harare (*H*, Dec. 96–Jan. 97) and Réunion (*R*, March-Apr. 97). Scoring on each individual plant was made on a 1–9 scale

ANMT, it was putative. It could thus be preferentially involved in early resistance. The QTL in bin 3.02 was detected for MSV35 in Réunion at 18 cM and by joint analysis of both environments at 2 cM. It was detected at a putative level only for late scoring dates (from 21 dai). In bin 3.03, a significant QTL was detected at 45 cM for APIT in Harare, whereas it was putative in Réunion. This region of the genome (comprising QTLs in bins

Table 2 (continued)

Variable	Place[a]	Chr	Bin and marker interval[b]	Centi Morgans[c]	LR[a]	*a*[e]	*d*[e]	R[f]	Action[g]
AUT	H	1	1.05	72	104.8	–1.22	–0.06	69.4	A
	R	1	asg30 (61 cM)	72	100.2	–1.47	0.04	67.0	A
	QTL×E	1	csu92 (85 cM)	72	39.7				
	H	10	10.05	62	31.7	–0.36	–0.50	29.8	OD
	R	10	npi232a (57 cM)	62	37.0	–0.52	–0.65	34.2	OD
	QTL×E	10	umc44a (69 cM)	62	18.6				
APIT	H	1	1.05	71	94.2	24.56	–3.58	64.1	PD
	R	1	asg30 (61 cM)	71	106.3	29.39	–6.47	74.7	PD
	QTL×E	1	csu92 (85 cM)	70	37.7				
	H	3	3.03	45	10.0	6.77	–1.11	4.9	PD
	R	*3*	*umc121 (11 cM)*	*45*	*9.9*	*7.24*	*–2.82*	*5.4*	*PD*
	QTL×E	*3*	*asg48 (45 cM)*	*45*	*2.9*				
	H	10	10.05	66	36.0	10.08	10.12	31.2	OD
	R	10	npi232a (57 cM)	65	35.6	10.90	11.46	30.1	OD
	QTL×E	*10*	*umc44a (69 cM)*	*65*	*2.9*				
ANMT	H	1	1.05	71	105.2	–1.02	0.28	78.8	PD
	R	1	asg30 (61 cM)	70	113.7	–1.24	0.49	87.9	PD
	QTL×E	1	csu92 (85 cM)	70	51.8				
	H	*2*	*2.03*	*53*	*7.4*	*–0.25*	*–0.04*	*4.4*	*PD*
	R	2	umc53a (19 cM)	53	9.0	–0.34	0.00	4.9	A
	QTL×E	*2*	*umc34 (71 cM)*	*62*	*4.1*				
	H	10	10.05	61	24.3	–0.20	–0.42	26.0	OD
	R	10	npi232a (57 cM)	62	28.1	–0.29	–0.54	29.5	OD
	QTL×E	10	umc44a (69 cM)	62	8.9				

[a] H, Harare; R, Réunion; QTL×E , QTL×environment interactions
[b] Bin estimated from the UMC 1995 reference map (Coe et al. 1995)
[c] Cumulative distance in centiMorgans (cM) from the first marker on the short arm of the chromosome to the position of the peak of the LR profile
[d] LR, likelihhood ratio
[e] *a* and *d*: Additive and dominance effects as estimated by the program at the peak of the LR profile, with model I. *d* has to be multiplied by 2 as it was estimated from F_3 families. Units: 1–9 scale for scoring variables, percentage of total number of plants in a plot for APIT. The sign of *a* indicates the origin of the allele contributing to the resistance: here all of these alleles come from the resistant parent (D211)
[f] R^2, Percentage of the phenotypic variation explained by the QTL under consideration
[g] Gene-type action, as described in the Methods. A, Additive; PD, partially dominant; D, dominant; OD, overdominant. Direction of the dominance is indicated by the sign of *d*

3.02/03) is likely involved in the late resistance. Localization of this QTL would be more accurate with a larger population. The putative QTLs in bins 1.09, 8.06, 9.01 were detected in Harare on specific dates for MSV07, MSV42, MSV14, respectively, whereas the putative QTLs in bins 2.05 and 2.09 were detected in Réunion for MSV35 and APIT, respectively. However, it is difficult to conclude whether they were preferentially involved in early or late resistance or specific to Harare as they were minor.

Some detected regions may be more involved in one of the resistance components, but this needs to be confirmed. On chromosome 2, the results suggest the presence of 2 minor QTLs, with the first, at around 60 cM, probably more involved in the severity component and the second, around 90 cM, more involved in the incidence resistance. The region in bin 3.03 may be more involved in the resistance to disease incidence as it was identified for APIT (incidence) but not for ANMT (severity).

The 2 QTLs with the largest proportion of explained phenotypic variation (R^2) were located in bins 1.05 and 10.05. In addition, the QTL in bin 3.09 explained early resistance as much as the QTL in bin 10.05. The major QTL on chromosome 1 (bin 1.05) explained from 47% to 72% of the variation for the disease scores. There were no clear variations in this percentage over time and/or locations. It explained about 68% of the phenotypic variation at both locations for the AUT variable. Its R^2 was higher for the ANMT variable (79% in Harare and 88% in Réunion) than for the APIT variable (64% in Harare and 75% in Réunion). The QTL on chromosome 10 (bin 10.05) explained between 21% and 34% of the phenotypic variation for all variables, including APIT and ANMT. The QTL in bin 3.09 explained a similar proportion of the phenotypic variation for the earliest scoring dates: 30% and 24% for MSV07 in Harare and Réunion, respectively. For MSV14, it accounted for about 20% of the phenotypic variation at both locations, even though it was detected at a putative level in Harare. Other QTLs explained around 10% or less of the phenotypic variation.

None of the possible types of gene action was favored. When the degree of dominance favors the resistance, we speak of dominance, when not, of recessiveness. The action of the major QTL in bin 1.05 appeared

Table 3 Genetic parameters associated with all QTLs, for scoring variables, and integration over time of these individual scores, of the proportion of symptom-free plants and of the mean score of plants presenting symptoms in a plot

Variable	Harare				Réunion			
	R^2 [a]	*a* [b]	*d* [e]	l2*d*/*a*l [d]	R^2	*a*	*d*	l2*d*/*a*l
MSV07	48	–0.26	0.01	0.09	55	–0.74	–0.03	0.07
MSV14	60	–0.96	–0.41	0.85	62	–2.44	–0.22	0.18
MSV21	61	–1.77	–0.67	0.76	62	–2.71	–0.83	0.61
MSV28	59	–2.49	–0.96	0.77	59	–2.79	–0.82	0.59
MSV35	60	–2.48	–0.99	0.79	62	–3.97	–1.52	0.77
MSV42	57	–2.55	–0.90	0.71	–	–	–	–
AUT	60	–1.58	–0.56	0.71	60	–1.99	–0.61	0.62
APIT	61	41.41	5.43	0.26	59	40.29	4.99	0.25
ANMT	59	–1.22	–0.14	0.23	61	–1.86	–0.05	0.06

[a] R^2, percentage phenotypic variation explained by all significant QTLs; obtained by regression on the flanking markers of these QTLs
[b] *a*, Global additive effect obtained by summing the additive effects of all significant QTLs detected for the variable in consideration;
[e] *d*, Global dominance effect obtained by summing the dominance effects of all significant QTLs;
[d] l2*d*/*a*l, degree of dominance in the F_2 generation

to be partially dominant for MSV07 and MSV14 in Harare, additive for all other scoring dates and AUT, and partially recessive for APIT and ANMT. The gene action for the other significant QTLs was overdominant (QTLs in bins 10.05 and 3.02 for MSV35), additive (QTL in bin 2.03), partially recessive (QTL in bin 3.03 for APIT), and overrecessive (QTL in bin 3.09). For putative QTLs, gene action was additive, except for QTLs in bin 8.06 and bin 9.01 for MSV14 where it was recessive and the QTL in bin 2.09 (overrecessive).

For all QTLs, except the putative ones in bins 1.09, 2.09, 8.06 and 9.01, alleles increasing the resistance were contributed by the resistant D211 parent.

QTL × environment interactions were tested for QTLs detected at least in one environment by joint analysis. Significant QTLs in bins 2.03 for MSV35 and ANMT, 3.02 for MSV35, 3.03 for APIT, and 3.09 for MSV14 were not detected by joint analysis, as was also the case for putative QTLs in bins 8.06 and 9.01. For the disease score variables, all QTLs but the putative one in bin 1.09 showed significant QTL × environment interactions. This was also the case for ANMT. For APIT, the region in bin 10.05 showed none of these interactions. This may correspond to non-significant genotype × environment interactions for disease incidence. Generally, low values for QTL × environment interactions corresponded to the mild genotype × environment interactions.

The range of phenotypic variation explained by all QTLs together for each variable (total R^2) varied from 43% to 61%. This total R^2 was similar for all locations, except for MSV07, where the percentages explained variation was higher in Réunion (Table 3). For all dates, these percentages were also similar, except at 7 dai, where R^2 was lower. Note that the sum of the individual QTL R^2 values was close to or over 100%, which is much higher than the total R^2 value obtained by multiple regression with all QTLs. This indicates that there were complementary effects among QTLs, even though calculating the R^2 for each QTL by estimating the genetic variance contributed by this QTL tended to give a higher value than that obtained by regression on the flanking markers of the considered QTL. This was especially true for the major QTL not tightly linked to its flanking markers.

The global gene action type was additive for the earliest scoring dates (except MSV14 in Harare), partially dominant thereafter. The resistance components (APIT and ANMT) showed a lower degree of dominance. It was even additive for ANMT in Réunion.

Evaluation with clones of different pathogenicity

To determine if there were any interactions between families and clones, and more precisely between some regions of the maize genome and some regions of the virus genome, we chose 32 $F_{2:3}$ families according to their genotype and phenotypic value. For genotype, all markers with an LR above 13.8 on chromosomes 1 and 10 were taken into account, plus 2 flanking markers on chromosome 10. Other regions, less stable over time or with a lower percentage of explained phenotypic variation, were not considered. As we were not sure whether the region on chromosome 1 and maybe also that on chromosome 10 involved 1 or 2 linked QTLs (Fig. 4), we decided to consider 2 linked QTLs, separated by the *asg30* hinge marker on chromosome 1, and the *npi232a* marker on chromosome 10. We then defined 16 "type groups", corresponding to the 16 possible combinations of these four "segments" of the genome in a homozygote state. If a marker was heterozygous, it was assumed to contribute to resistance as much as the favorable allele (from parent A in the considered regions), since the action of the considered region on chromosome 10 was overdominant, that of the region on chromosome 1 was additive, and dominance was never in favor of susceptibility. When the allelic origin of a segment was mainly from the resistant parent, this segment was denoted A, otherwise it was denoted B. When possible, 2 families were assigned to each group type. If some markers were heterozygous, there were preferably

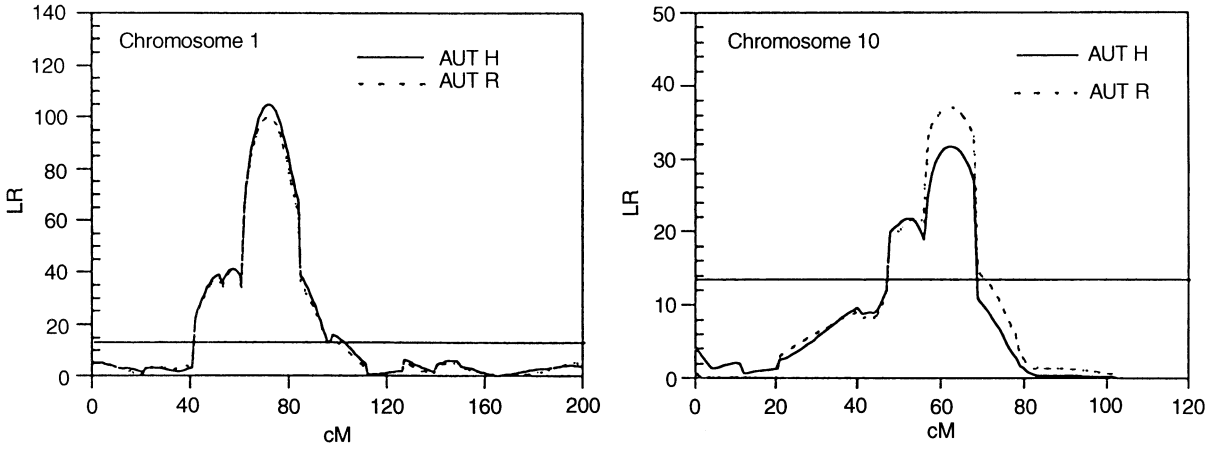

Fig. 4 QTL likelihood profiles indicating LR values for AUT in Harare (*H*) and Réunion (*R*) on chromosomes 1 and 10. The *horizontal line* indicates the level of significance at LR=13.8 (equivalent to a LOD of 3). The LR score was calculated every 1 cM

not the same ones in both families. The phenotypic values of these families for AUT, APIT and ANMT in both environments represented the full range covered by the entire population.

The artificial inoculations in cages were successful: the susceptible checks were all dead at 28 dai, and no significant differences between 2 cages for the same clone in the same replication were noted (data not shown). The family genotype effect was highly significant for all considered traits, for each separate experiment with the four clones (data not shown), and when the whole trial was considered (Table 4). The clone effect was significant for all scoring dates but MSV28. When considering the resistance components, the effect was significant at all dates for incidence, but only at 9 dai for severity (Table 4). Clone × genotype interactions were significant for incidence at all dates and for severity at 14 and 28 dai. They were not significant for any of the scoring variables (Table 4). In this experiment, "clones" differed significantly in terms of their pathogenicity (capability of causing disease, related to the overall resistance), of their virulence (capacity to produce symptoms in a genotype, linked to disease incidence), but not of their aggressiveness (quantity of symptoms, linked to disease severity). By these definitions, virulence and aggressiveness are two components of pathogenicity (Fraser 1986).

According to the Newman-Keuls test results (Table 4), when the incidence variables were considered, means for the four "clones" could be pooled into three groups. As expected, R4 (chosen for its mildness) was the least virulent of the four "clones", while R2 was the most virulent. RX and the R2 and R4 co-inoculation formed an intermediate group. In this case, there was no complementation between the two R2 and R4 clones since co-

Table 4 *F* tests from analysis of variance combined across clones and means of scoring (MSVi), incidence (PISi) and severity variables (NMSi) at the ith day after infestation (obtained in Dec. 96-Jan. 97 in Réunion)

F test							Means of clones[a]				
Source	Clone	Rep/clone	Block/ (rep × clone)	Genotype	Clone × genotype	Residual SD	R2	R4	RX	R2+R4	Mean
df	3	4	8	31	93	116					
MSV09[b]	30.57***	38.78***	2.06*	47.35***	0.67	0.27	2.51a	2.06c	2.32b	2.33b	2.31
MSV14	9.42***	0.48	1.49	146.55***	1.05	0.30	3.35a	3.07c	3.21b	3.16bc	3.20
MSV21	8.62***	1.13	1.22	165.92***	1.23	0.31	3.38a	3.14b	3.28a	3.37a	3.29
MSV28	2.52	4.22**	0.87	217.12***	1.20	0.29	3.26a	3.18a	3.15a	3.26a	3.21
PIS09	39.66***	4.17**	1.10	56.11***	2.01***	10.40	10.78c	30.65a	22.99b	21.02b	21.36
PIS14	41.04***	0.69	0.81	49.86***	2.52***	10.14	8.00c	27.64a	19.57b	16.45b	17.92
PIS21	41.90***	0.53	0.72	53.44***	2.66***	10.00	8.22c	27.80a	19.71b	16.66b	18.10
PIS28	22.97***	1.37	1.29	113.93***	1.80**	9.22	20.52c	33.73a	28.35b	25.48b	27.02
NMS09	13.13***	27.29***	1.20	16.37***	1.14	0.34	2.62a	2.27b	2.55a	2.53a	2.49
NMS14	1.50	1.17	1.19	93.47***	1.47*	0.32	3.44a	3.38a	3.45a	3.34a	3.40
NMS21	0.94	0.69	1.01	86.75***	1.31	0.38	3.48a	3.42a	3.51a	3.52a	3.48
NMS28	0.04	4.04**	1.49	102.74***	1.40*	0.42	3.41a	3.39a	3.40a	3.42a	3.41

*, **, *** Significant at the 0.5, 0.01, and 0.001 probability levels respectively

[a] Means in same line, followed by same letter are not significantly different at the 0.05 probability level (Newman-Keuls' test)

[b] Variables: MSVi, individual scoring variable at i days after infestation (1=no symptoms, 9=dead); PISi, proportion of symptom-free plants in a plot at date i; NMSi, mean scoring of the plants showing symptoms in a plot at the date i

inoculation led to an intermediate level of virulence relative to that of each component. When considering the scoring variables, clone means were classified in the same way. The R2 clone was always the most pathogenic with the R4 clone being the least. When considering NMS09, *i.e.* the only severity variable affected by a clone effect, the R4 clone was the least aggressive, while the three other "clones" were not significantly different. In this case, the complementation hypothesis was not invalidated.

The existence of clone × genotype interactions suggested a specific relationship between some genotypes and clones. However, this specificity could not be characterized at the genetic level. Genotypes with all four resistant alleles in the studied regions were the most resistant ones, regardless of the clone. Hence, depending on the allelic combination at these four genomic segments, and at all other loci not taken into account, the genotypes were more or less resistant without any clearly repeated structuring throughout the trials with the four different clones. Moreover, with the clones used, most of the not totally resistant genotypes did not differ significantly from each other (data not shown).

Discussion

Control of resistance in the D211xB73 population

Lines with complete resistance to maize streak virus, including D211 and CIRAD390, were selected at CIRAD (Rodier et al. 1995; Clerget et al. 1996). Genetic control of this resistance was studied by generation mean analysis of six generations of the cross between the resistant D211 and the susceptible B73 inbred lines (Rodier 1995). Our study aimed primarily at specifying the results of the genetic analysis, and locating the QTLs in the genome. Moreover, we investigated the response of F_3 families to clones of different pathogenicity.

One important prerequisite in QTL mapping experiments is to accurately estimate the genotypic value of each individual of the population. The use of insects selected for a 100% transmission rate and the control of environmental heterogeneity by using an experimental design with two repetitions in two different environments helped us to fulfill this primary requirement, as shown by the high heritability values.

The physiological age of the plants at the time of infestation was younger in Réunion than in Zimbabwe. This age difference, in addition to the pathogenicity of the MSV isolates, could explain the difference between the two locations. Within a location, plants also did not have exactly the same physiological age at the time of infestation. Nevertheless, genotypic variability in MSV resistance was tremendous between the F_3 families. This issue of physiological age should be taken into account in more specific studies on resistance mechanisms.

The major hypothesis in using the composite interval mapping model described by Zeng (1994) is the absence of epistasis. If epistasis is present, the consequence is that the QTL detected in the interval bracketed by the two neighboring markers is not completely independent of QTLs located elsewhere in the genome. However, the detection accuracy obtained using this model with cofactors is still higher than with a simple interval mapping model, because of the reduced residual genetic variance. Tanksley and Nelson (1996) put forward the hypothesis that epistatic effects could be very important in balanced populations between a non-adapted line and an elite line, which was the case in our population. Rodier (1995, personal communication) demonstrated the existence of non-allelic interactions. Epistasis would be of the duplicate type, with the homozygote × homozygote component favoring resistance, but not the heterozygote × heterozygote component. However, these effects contributed much less to genetic variation than the additive and dominant effects. QTL detection could only be slightly biased, if at all.

In our study, we confirmed that MSV resistance was quantitatively inherited. We detected at least 5 significant QTLs on chromosomes 1, 2, 3 and 10, which explained between 48% and 62% of the total phenotypic variation, depending on the variables studied. Complementary effects among QTLs probably exist. In spite of the use of linked cofactors, we could not determine whether the QTL on chromosome 1 involved just one genetic factor or was a linkage of several of them (Fig. 4). Larger populations should be analyzed and more markers placed in these regions in order to decide whether there is a linkage of genetic factors or whether the peaks are just due to high bias in estimating mean and residual variance between 2 neighboring markers (van Ooijen 1992). Another solution would be to develop an advanced intercross line population in order to break down the linkage, as proposed by Darvasi and Soller (1995).

These QTLs were stable for all dates except those on chromosome 3, where one region seemed to be more involved in early resistance, and the other in later resistance. Some variation in the action type could be identified in some cases. As no confidence interval was established for the estimates of parameters *a* and *d*, we could not determine whether there was a contingent lack of statistical accuracy or a genetic cause. We thus cannot overlook a possible action of some minor modifier genes not detected in this experiment. Moreover, we should point out that the action type of the chr1-QTL was partially recessive for the two resistance components APIT and ANMT, whereas it was additive for AUT. These results enabled us to determine the best time for screening for MSV resistance. At this date, most of the genetic factors should best express their own respective effects. The date should be not too late after infestation, for two reasons: (1) the genetic variation explained by the QTL on chr1 may mask the effects of the other minor QTLs, and (2) there is a lack of scoring accuracy because many other biotic or abiotic stresses may interfere with MSV resistance. Based on these criteria, we propose scoring

MSV resistance 14 dai and then confirming this first notation 35 dai. As screening for MSV resistance is quite straightforward, marker-assisted selection will be more powerful than conventional selection only if this resistance needs to be combined with other traits that are difficult to evaluate simultaneously.

QTLs were stable across the two environments, which means that selection for MSV-resistant varieties may be efficient without multilocation tests. However, QTL × environment interactions were mild but frequent. These QTL × environment interactions may be partly responsible for the changes between environments in the type of gene response actions. One possible cause, apart from the infestation conditions, may be a variability in isolates between the two locations: there is probably a difference in their pathogenicity either because of different pathogenic determinants or because of their mode of conservation, which plays a role in their composition as shown by Isnard (1998). Genetic host-pathogen relationships were investigated in the second part of that study.

The genetic foundations of the disease incidence (APIT) and severity (ANMT) seemed to be slightly different, as no significant QTLs were detected for APIT on chromosome 2, and 1 QTL on chromosome 3 was not identified for ANMT (even as putative). However, these QTLs were minor ones. The existence of 2 QTL sets (partly different) for these two traits should be investigated further before postulating on the existence of different resistance mechanisms.

These results closely agree with those obtained by Rodier (1995) who used the generation mean analysis method (Mather and Jinks 1982). According to this method, the D211 resistant line would have at least one to two genetic factors with the Castle and Wright formula (Castle and Wright 1921) and two to three with the Mather and Jinks formula. The number of genes was likely underestimated by these formulas, as epistasis was detected. Rodier et al. (1995) also proposed that minor genes may be involved. In other resistance sources, 1 major QTL was mapped on chromosome 1 (Kyetere et al. 1999; Welz et al. 1998). This quantitative trait allele may be identical or allelic to the one mapped in D211. Minor QTLs were also identified by Welz et al. (1998) but not on the same chromosome marker intervals as in D211.

Stability of the resistance against different MSV clones

There is variability at the molecular level between MSV isolates from different African countries (Briddon et al. 1994; Isnard et al. 1997; Ngwira et al. 1997). One should wonder whether the same resistance genes are efficient everywhere MSV epidemics occur. In a multilocation trial, MSV resistance of varieties from Réunion was shown to be stable (Dintinger et al. 1997). However, in the first part of that study, genotype × environment interactions were noted. It was hypothesized that isolate variability may be partly responsible for these interactions. In order to determine whether there were any genotype × clone interactions, or even QTL × clone interactions, we carried out the present study by testing families with different sets of QTLs against MSV clones that were previously shown to be of different pathogenicity (Isnard 1998).

In our study, the three clones R2, R4, RX differed in terms of their pathogenicity, especially their virulence. They differed significantly in their aggressiveness only at a very early stage of resistance. It was impossible to determine whether the number of genetic factors needed for resistance depended on the level of pathogenicity of the clone, for at least two reasons: (1) the genetic noise, due to all of the other genetic factors not taken into account, and (2) the heterozygosity of some of the considered markers. Genotype × clone interactions were significant, especially for the incidence variables. QTL × clone interactions could not be defined. However, the existence of genotype × clone interactions suggests some specific relationships between a portion of the maize genome and the genetic determinants of the clonal pathogenicity. One point in favor of this hypothesis is that some symptom regressions were noted, more or less marked, depending on the genotype – clone combination (data not shown). Moreover, the fact that these interactions existed for the incidence variables, sometimes also for the severity variables, but never for the disease scoring variables, indicated that two different resistance mechanisms may have been present.

In a study on maize chlorotic dwarf virus (MCDV), Pratt et al. (1994) demonstrated that a severe isolate induced more severe symptoms than a regular isolate. They also found some host genotype × isolate interactions (9 genotypes and 2 isolates), but only for the mean symptom rating (taking into account both the disease incidence and severity) and not for the mean disease incidence, *i.e.* contrary to our case where these interactions were stronger when considering the disease incidence. Nonetheless, these results do not contradict the hypothesis of specific interactions between some regions of the maize genome and some specific determinants of MSV or MCDV clones.

The existence of these interactions implies that selection should be focused on completely resistant genotypes, which were stable regardless of the clone, or selection processes could be conducted in different areas where streak disease occurs. In order to confirm and specify the nature of the interactions, the experiment should be repeated with more clones with known sequences, varying in their pathogenicity, and with maize isolines, representing various combinations of MSV resistance alleles. It would be also useful to specify the number and the location of resistance factors in D211 as well as in other resistant lines. In a companion study, a second population segregating for resistance to MSV has been mapped. The susceptible parent is still B73, while the resistant parent is CIRAD390, another resistant line from Réunion. In that study, a detailed comparison with the identified resistance factors located in other resis-

tance sources (Kyetere et al. 1999; Welz et al. 1998) was undertaken in order to examine their stability throughout germplasm. The results of all of these studies should allow us to discuss the durability of the Réunion complete polygenic resistance on a stronger basis.

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