Theor Appl Genet (1999) 99:540–553

A. Pernet · D. Hoisington · J. Dintinger · D. Jewell
C. Jiang · M. Khairallah · P. Letourmy
J.-L. Marchand · J.-C. Glaszmann
D. González de León

Genetic mapping of maize streak virus resistance from the Mascarene source. II. Resistance in line CIRAD390 and stability across germplasm

Received: 15 June 1998 / Accepted: 30 January 1999

Abstract The streak disease has a major effect on maize in sub-Saharan Africa. Various genetic factors for resistance to the virus have been identified and mapped in several populations; these factors derive from different sources of resistance. We have focused on the Réunion island source and have recently identified several factors in the D211 line. A second very resistant line, CIRAD390, was crossed to the same susceptible parent, B73. The linkage map comprised 124 RFLP markers, of which 79 were common with the D211×B73 map. A row-column design was used to evaluate the resistance to maize streak virus (MSV) of 191 $F_{2:3}$ families under artificial infestation at two locations: Harare (Zimbabwe) and in Réunion island. Weekly ratings of resistance were taken and disease incidence and severity calculated. QTL analyses were conducted for each scoring date and for the integration over time of the disease scores, of incidence, and of severity. Heritability estimates (71–98%) were as high as for the D211×B73 population. Eight QTLs were detected on chromosomes 1, 2, 3, 5 (two QTLs), 6, 8, and 10. The chr1-QTL explained the highest proportion of phenotypic variation, about 45%. The QTLs on chromosomes 1, 2, and 10 were located in the same chromosomal bin as QTLs for MSV resistance in the D211×B73 population. In a simultaneous fit, QTLs explained together 43–67% of the phenotypic variation. The QTLs on chromosomes 3, 5, and 6 appeared to be specific for one or the other component of the resistance. For the chr3-QTL, resistance was contributed by the susceptible parent. There were significant QTL × environment interactions for some of the variables studied, but QTLs were stable in the two environments. They also appeared to be stable over time. Global gene action ranged from partial dominance to overdominance, except for disease severity. Some additional putative QTLs were also detected. The major QTL on chromosome 1 seemed to be common to the other sources of resistance, namely Tzi4, a tolerant line from IITA, and CML202 from CIMMYT. However, the distribution of the other QTLs within the genome revealed differences in Réunion germplasm and across these other resistance sources. This diversity is of great importance when considering the durability of the resistance.

Key words Quantitative trait loci · Resistance mechanism · MSV · Composite interval mapping · Tropical maize

Communicated by G. Wenzel

A. Pernet · D. Hoisington · C. Jiang · M. Khairallah
D. González de León
CIMMYT, Apdo Postal 6–641, 06600 Mexico D. F., Mexico

A. Pernet (✉) · P. Letourmy · J.-L. Marchand · J.-C. Glaszmann
CIRAD, 2477 avenue Agropolis, BP 5035,
34032 Montpellier cedex 1, France
Fax:+33 4 67 61 56 05

D. Jewell
CIMMYT, P.O. Box MP154, Mount Pleasant, Harare, Zimbabwe

J. Dintinger
CIRAD-CA, Station de Ligne Paradis, 7 chemin de l'IRAT,
97410 Saint Pierre, Réunion island

Introduction

Streak disease is caused by the maize streak virus (MSV), a geminivirus transmitted only by leafhoppers of the genus *Cicadulina*. It is widespread in Africa south of the Sahara and in the Mascarene islands of the Indian Ocean. It has provoked very serious damage on maize crops, including total yield loss. Several studies aimed at understanding the genetic basis of resistance to MSV have been undertaken using either quantitative genetics or molecular mapping.

The detection and localization of resistance factors to MSV were conducted in two different populations, for which the source of resistance was either of Nigerian origin (Kyetere et al. 1999) or unknown for the CIMMYT inbred line CML202 (Welz et al. 1998). In both cases, one major quantitative trait locus (QTL) was found on chromosome 1. In CML202, some minor QTLs were also detected

on chromosomes 2, 3, and 4 for the earliest disease scoring date. One population whose resistance came from the composite CVR3-C3 obtained by intermating 41 Mascarene populations and a South African line in Réunion island (Marchand et al. 1994) was also mapped. Some families of this D211 × B73 population were tested against clones of different pathogenicity (Pernet et al. 1999). A major QTL on chromosome 1 was identified. Other QTLs were detected on chromosomes 2, 3, and 10. There were genotype × virus clone interactions, especially for disease incidence.

In order to increase the scope of our survey of resistance factors present in the initial Réunion composite, we conducted a genetic mapping experiment using a cross that involved another completely resistant line from Réunion, CIRAD390, and the same susceptible parent. The objectives were (1) to identify genomic regions responsible for resistance to MSV and estimate their genetic effects with the composite interval mapping method; (2) to determine if some genetic factors were specifically involved in one or the other of the two resistance components studied (severity and incidence), and/or preferably in the expression of resistance at earlier or later stages; (3) to elucidate whether different genetic factors conferring resistance to MSV exist in the CIRAD germplasm and compare these with QTLs mapped in previous studies.

Materials and methods

Plant material and experimental design

The F_2 population developed for this study originated from a cross between CIRAD390, the MSV resistant parent, and B73, the susceptible one. CIRAD390 (S_3 generation) was selected in Réunion island for complete resistance to MSV (Clerget et al. 1996). The local donor of resistance, CVR3-C3, was crossed to population Tocumen 7931 and then backcrosssed twice to the population Suwan 8331. The CIRAD390 line was then extracted at the S_1 generation. B73 is an American inbred from the Stiff-Stalk group. The material was produced as for the D211×B73 population (hereafter called DB) described in the companion paper (Pernet et al. 1999). The population CIRAD390×B73 (hereafter called CB) comprised 200 F_2 individuals derived from one F_1 plant. From their F_2 individuals, 191 $F_{2:3}$ families yielded sufficient seed quantities for the replicated trials.

Trials were conducted in two different locations: Harare (CIMMYT station, Zimbabwe), and Saint Pierre (CIRAD station, Réunion). In addition to the parental lines, different checks were used, depending on the location: Kilima S4–8, Kilima S4–12 as resistant checks and CG4141 as a susceptible check in Harare; the hybrid Sabrina from Pioneer France Maïs as susceptible in Réunion. The 191 $F_{2:3}$ families, both parents, and six susceptible and five resistant checks fitted in a 17×12 row-column design with two replications (John and Eccleston 1986) planted on Nov. 28, 1995 and Feb. 15, 1996, in Harare and in Réunion, respectively. Plots were oversown, by hand, with two seeds per hill. Plants were randomly thinned to 7 plants per square meter before infestation in Réunion and after infestation in Harare.

Resistance evaluation

Disease assesment

Artificial infestation was done along the plant lines (rows of the design) using standard methods developed at IITA (Leuschner et al. 1980) with the viruliferous insect vector *C. mbila*. Inoculations were made at the three- to four-leaf stage in Harare (Dec. 14, 1995), and two- to three-leaf stage in Réunion (Fev. 28, 1996). Isolates were those usually used for selection in the stations where the trials were conducted. Genetic heterogeneity of these isolates is highly probable (Isnard et al. 1997).

Symptoms were evaluated on the last fully expanded leaf of each plant on a 1 (resistant, no symptoms at all) to 9 (susceptible, leaf fully chlorosed, plant almost dead) scale once per week until 42 days after infestation (dai). This scale is correlated to the proportion of chlorotic area of the leaf, to the virus concentration in the leaf, and to the chlorophyll concentration (Rodier 1995).

Variable description

The resistance to MSV and to two of its components were studied as for the DB population (Pernet et al. 1999). In addition to the disease score given to each individual plant at the u^{th} dai, designated MSV_u, the number of plants free of symptoms was recorded per plot. The variable PIS_u is then the proportion of symptom-free plants per plot and represents the disease incidence. The severity of the disease, NMS_u, was calculated as the mean disease 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. This variable NMS_u was renamed NMX_u when these families showing complete resistance were excluded from the analyses of the disease severity. In order to integrate the value of these variables over time, we calculated the area under the disease progress curve (AUDPC): this was called AUT for the score, APIT for incidence, and ANMT and ANMX for severity, with or without the families showing complete resistance, respectively, according to the general formula given by Ceballos et al. (1991). The sixth scoring was taken at 51 dai for the trial in Réunion. The total length of time was thus 51 dai for that trial and 42 dai for the trial in Harare.

Restriction fragment length polymorphism (RFLP) genotyping

Protocols for RFLPs were the same as in the DB population. The same set of probes was used for the parental screening (Pernet et al. 1999). The best probes were hybridized onto the whole population. These were chosen in order to provide a uniform coverage of the genome and at the same time make links with the DB population. Genetic data were captured and verified by two different readers using HyperMapData software (Hoisington et al. 1993).

Data analyses

Map construction

Possible distortion segregation with respect to the expected Mendelian proportions, was determined at each marker locus. The genetic map, based on the 200 individuals, was constructed using the software MAPMAKER 2.0 (Lander et al. 1987) following the same procedure as in Pernet et al. (1999).

Field data

Analyses of variance were conducted on a plot-mean basis or on an individual basis, depending on the variable, within each environment using the SAS Mixed procedure (SAS 1997). All factors were assumed to be random variables. Normality of the distribution of the residual errors was examined using the SAS Univariate procedure (SAS 1997). BLUPs (best linear unbiased predictors, Henderson 1975) were obtained by adding the general mean of the trial to the solution of the random "Genotype" effect. Broad-sense heritabilities at the experimental design level were calculated overall for both environments, with location effect considered as fixed, and genotype × environment interactions as random. For more details, see Pernet et al. (1999).

QTL analyses

Methodology of detection

QTL analyses were conducted following the same methodology as for the analyses with the DB population (Pernet et al. 1999). The multiple trait analysis method described by Jiang and Zeng (1995), based on the composite interval mapping method (Zeng 1994), allowed the estimations of genetic effects, such as additivity, dominance, pleiotropy, and QTL × environment interactions. These QTL analyses were performed on the families for which both phenotypic and genotypic data were collected. When the variable ANMX was studied, 181 families remained. As defined by Zeng (1994), BLUPs of each trait were analyzed using three sub-models successively: without any cofactor (model III), with unlinked markers as cofactors (model II), and with unlinked and linked markers to the region being tested as cofactors (model I, window size of 20 cM). For more details on the choice of cofactors, see the companion paper (Pernet et al. 1999). Cofactor sets were combined when several traits were analyzed at the same time.

Determination of the threshold

A QTL was declared significant when the LOD (decimal logarithm of the likelihood odds ratio) was above 3.0, either with model I or model II, a peak being also identified with model I. A QTL was declared putative when the LOD value was between 2.0 and 3.0 with model I and not above 3.0 with model II. This lower threshold allowed the consistency over the two environments for some QTLs to be reflected and the putative QTLs of the CB population to be compared with those of the DB population studied previously. When conducting joint analysis of two traits at the same time, the LR (log likelihood ratio) threshold had to be above 17.8 with model I for declaring a "joint" QTL significant (Zeng 1994; Jiang and Zeng 1995). In chromosomal regions where a QTL was detected by joint analysis, QTL × environment interactions were tested and declared significant for an LR value above 5.99 (Jiang and Zeng 1995). Estimate of the QTL position was given by the LOD-curve peak in model I. Additive and dominance effects (*a* and *d*, respectively) for each QTL were estimated by regression under model I. The dominance ratio (DR=|2*d*/*a*|) characterized the type of gene action: additive for DR<0.2, partially dominant for 0.2=DR<0.8, dominant for 0.8=DR<1.2, and overdominant for DR=1.2 (Stuber et al. 1987). *d* had to be multiplied by 2, as it was estimated from F_3 families. All results are given using the value of the LR, which is the standard output of the program. LR values of 13.8, 11.5, 9.2 correspond to LODs of 3.0, 2.5, 2.0, respectively.

A QTL identified in the CB population was considered to be identical to one identified in the DB population if both were located in the same chromosomal bin of the UMC 1995 linkage map (Coe et al. 1995) and if flanking markers presented the same RFLP allele.

Proportion of the variation explained

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

Results

RFLP linkage map

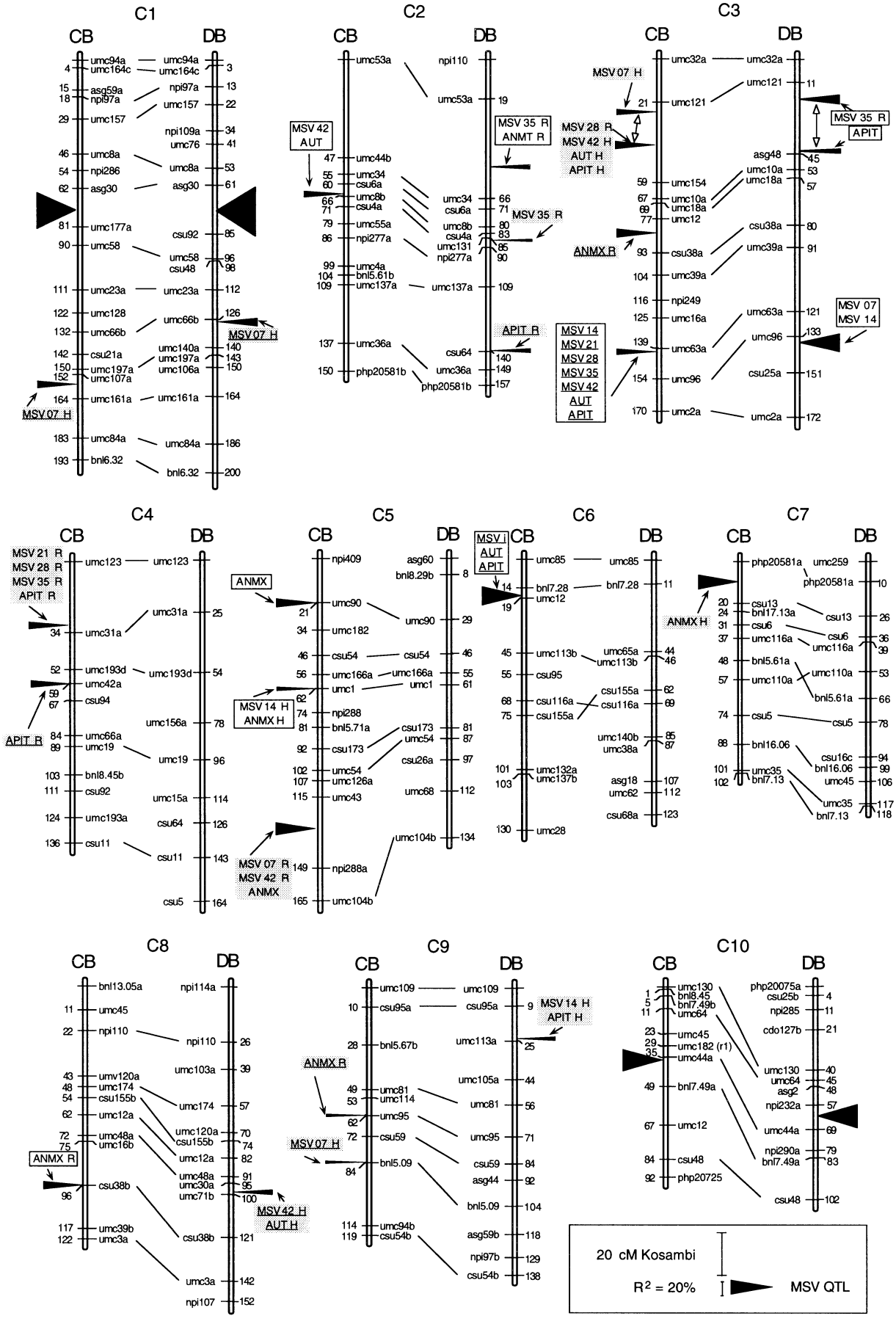
Of the RFLP probes used on the parental lines 86% detected polymorphism between the parents, a percentage similar to that obtained for the DB population. Mostly codominant markers, well spread over the genome and segregating in the two populations, were selected for genotyping the F_2 individuals. Some loci which formed a cluster with other markers were discarded. When the sequentially rejective Bonferroni procedure (Holm 1979) was used, none of these markers showed significant segregation distortion.

The final map comprised 124 markers (103 codominant and 21 dominant) on ten linkage groups spanning 1379 cM (Fig. 1). The *umc53a* distal marker was attributed to chromosome 2, as in the 1995 UMC reference map (Coe et al. 1995), although it was linked only at LOD 2.5 to the closest marker of this chromosome. The order of the markers was in good agreement with the reference map. The average distance between 2 markers was 12.1 cM, which is similar to that obtained for the DB population, and the standard deviation was 7.9 cM. Seventy-nine probes were common to the two populations. The good coverage of the genome by the maps allowed an extensive search for the QTLs and a precise comparison between the two populations.

Field trait analyses

The success of the artificial inoculations was assessed on the susceptible checks (Fig. 2). B73 was almost completely dead in all trials, at the latest by 35 dai. The resistant parent CIRAD390 exhibited some chlorotic spots on some individuals (score 2) between 14 and 28 dai, whereas the resistant parent D211 did not shown any symptoms. However, these spots were not observed in the latest scorings.

General statistical descriptors were close to those obtained for the DB population. Normality of the residual distributions was met for the variables PIS14 and PIS21 in both locations and for NMS21, APIT and ANMT in Harare. However, the distribution was symmetrical for the other variables, except MSV07 in Harare. Genetic variability was high for all traits at both locations (Table 1). Heritabilities ranged from 71% (MSV07) to 98% (MSV28, MSV35, MSV42), increasing with time after infestation. The genotype × environment (G×E) interaction variance component ($\hat{\sigma}^2_{G\times E}$) was not significant for the latest disease scores (MSV28, MSV35, MSV42, and AUT) in contrast to what had been observed for the DB population. $\hat{\sigma}^2_{G\times E}$ was significant for a few severity variables (it was significant for all of the severity variables but NMS07 for the DB population) and for some incidence variables (which was not the case for the DB population).


C1
CB
DB
umc94a
umc164c
asg59a
npi97a
umc157
umc8a
asg30
umc177a
umc58
csu48
umc23a
umc128
umc66b
csu21a
umc197a
umc107a
umc161a
umc84a
bnl6.32
umc94a
umc164c
npi97a
umc157
npi109a
umc76
umc8a
asg30
csu92
umc58
umc23a
umc66b
umc140a
umc197a
umc106a
umc161a
umc84a
bnl6.32
MSV 07 H
MSV 07 H
C2
CB
DB
umc53a
umc44b
umc34
csu6a
umc8b
csu4a
umc55a
npi277a
umc4a
bnl5.61b
umc137a
umc36a
php20581b
npi110
umc53a
umc34
csu6a
umc8b
csu4a
umc131
npi277a
umc137a
csu64
umc36a
php20581b
MSV 42 AUT
MSV 35 R ANMT R
MSV 35 R
APIT R
C3
CB
DB
umc32a
umc121
umc154
umc10a
umc18a
umc12
csu38a
umc39a
npi249
umc16a
umc63a
umc96
umc2a
umc32a
umc121
asg48
umc10a
umc18a
csu38a
umc39a
umc63a
umc96
csu25a
umc2a
MSV 07 H
MSV 28 R
MSV 42 H
AUT H
APIT H
ANMX R
MSV 35 R
APIT
MSV 14
MSV 21
MSV 28
MSV 35
MSV 42
AUT
APIT
MSV 07
MSV 14
C4
CB
DB
umc123
umc31a
umc193d
umc42a
csu94
umc66a
umc19
bnl8.45b
csu92
umc193a
csu11
umc123
umc31a
umc193d
umc156a
umc19
umc15a
csu64
csu11
csu5
MSV 21 R
MSV 28 R
MSV 35 R
APIT R
APIT R
C5
CB
DB
npi409
umc90
umc182
csu54
umc166a
umc1
npi288
bnl5.71a
csu173
umc54
umc126a
umc43
npi288a
umc104b
asg60
bnl8.29b
umc90
csu54
umc166a
umc1
csu173
umc54
csu26a
umc68
umc104b
ANMX
MSV 14 H
ANMX H
MSV 07 R
MSV 42 R
ANMX
C6
CB
DB
umc85
bnl7.28
umc12
umc113b
csu95
csu116a
csu155a
umc132a
umc137b
umc28
umc85
bnl7.28
umc65a
umc113b
csu155a
csu116a
umc140b
umc38a
asg18
umc62
csu68a
MSV i
AUT
APIT
C7
CB
DB
php20581a
csu13
bnl17.13a
csu6
umc116a
bnl5.61a
umc110a
csu5
bnl16.06
umc35
bnl7.13
umc259
php20581a
csu13
csu6
umc116a
umc110a
bnl5.61a
csu5
bnl16.06
csu16c
umc45
umc35
bnl7.13
ANMX H
C8
CB
DB
bnl13.05a
umc45
npi110
umv120a
umc174
csu155b
umc12a
umc48a
umc16b
csu38b
umc39b
umc3a
npi114a
npi110
umc103a
umc174
umc120a
csu155b
umc12a
umc48a
umc30a
umc71b
csu38b
umc3a
npi107
ANMX R
MSV 42 H
AUT H
C9
CB
DB
umc109
csu95a
bnl5.67b
umc81
umc114
umc95
csu59
bnl5.09
umc94b
csu54b
umc109
csu95a
umc113a
umc105a
umc81
umc95
csu59
asg44
bnl5.09
asg59b
npi97b
csu54b
ANMX R
MSV 07 H
MSV 14 H
APIT H
C10
CB
DB
umc130
bnl8.45
bnl7.49b
umc64
umc45
umc182 (r1)
umc44a
bnl7.49a
umc12
csu48
php20725
php20075a
csu25b
npi285
cdo127b
umc130
umc64
asg2
npi232a
umc44a
npi290a
bnl7.49a
csu48
20 cM Kosambi
R² = 20%
MSV QTL

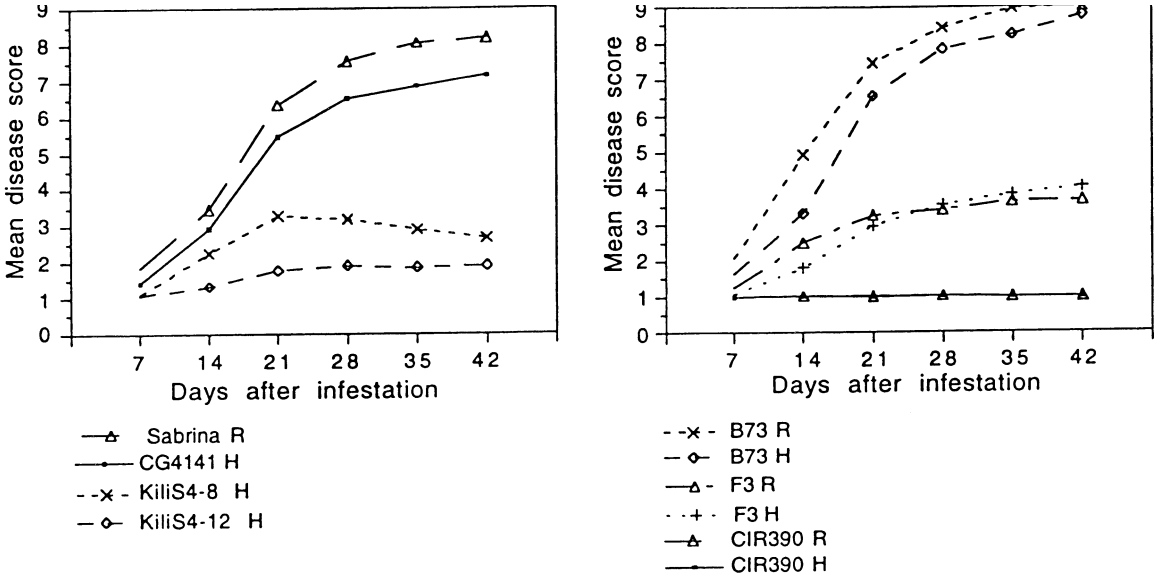


Fig. 2 Time-course of the disease symptoms on the susceptible checks (CGR4141 in Harare, Sabrina in Réunion), on the resistant checks (Kilima S4–12 and Kilima S4–8 in Harare, CIRAD390, in Réunion), on the susceptible parent B73, on the resistant parent CIRAD390 and on the $F_{2:3}$ families in Harare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

G×E interactions related to disease incidence for the CB population were probably due to a difference between the locations in the infestation process. Namely, for the CB trial in Réunion, variance between the lines of infestation (i.e. represented by the rows of the design) was significant for scoring variables (MSV07, MSV14, MSV21) and some incidence variables (PIS07, PIS14, and APIT) but not for the severity variables (NMS_u and ANMT), whereas neither the variance between rows nor the variance between columns were significant for any variable in the CB trial in Harare (results not shown). The variance between the rows of the CB trial in Réunion may be due to the fact that infestation was done during a windy period for some rows; this could have hampered the infestation of some plants (disease incidence) by the insects. However, natural secondary infestations occurred later since differences between lines disappeared after more than 14 dai.

The distribution of $F_{2:3}$ families for the various variables analyzed indicates that resistance to MSV is quantitatively inherited in CIRAD390 (Fig. 3). No transgression could be detected for the F_3 families. The percentage of very resistant plants (Fig. 3) was higher than for the DB population. Consequently, at both locations, disease incidence was lower for the CB population, whereas disease severity (ANMT) appeared not to be different between the two populations within the same environment. CB and DB populations may thus display different genetic determinants.

◀ **Fig. 1** RFLP linkage maps with location of QTLs for MSV resistance in populations CIRAD390×B73 (*CB*) and D211×B73 (*DB*). Cumulative distances are in centi Morgans on the *left* (*right*) of each maize chromosome, for the CB population (for the DB population). Common markers to the two maps are linked by a *black line*. *Peaks 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 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

QTLs in the CIRAD390×B73 population

Eight significant QTLs, with an LR value above 13.8 (LOD=3.0) were identified for this population, in bins 1.05, 2.04, 3.09, 5.02, 5.03, 6.01, 8.07, and 10.06. Nine regions distributed over six chromosomes were detected with an LR value equivalent to LOD 2–3 (putative QTLs). They were located in bins 1.10, 3.02 (one or two QTLs), 3.06, 4.02, 4.05, 7.01, 9.05, 9.06 and on the long arm of chromosome 5 (chr5L) (Fig. 1). One more region was detected only by joint analysis of both environments, on chromosome 4, around 78 cM (bin 4.06) for MSV21 and MSV42 (data not shown).

The localization of the significant QTLs on chr1 and chr10 was consistent across environments and dates. Other significant QTLs were also consistent over the two environments. The QTL in bin 2.04 (65 cM) was significant for the variables MSV42 and AUT in Réunion and putative for these variables in Harare. In Réunion, this

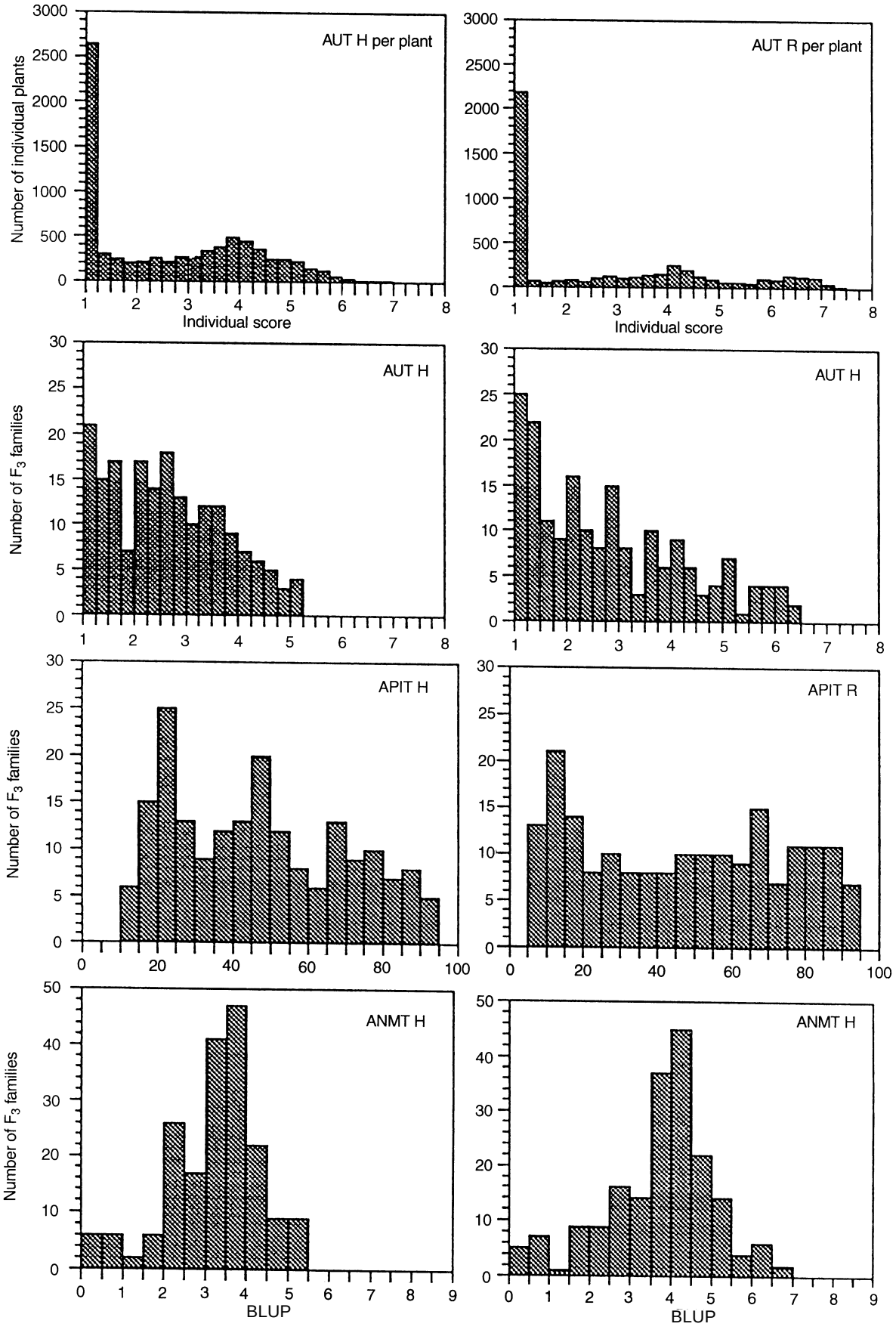

AUT H per plant
AUT R per plant
AUT H
AUT H
APIT H
APIT R
ANMT H
ANMT H
Number of individual plants
Number of F_3 families
Individual score
BLUP

Table 1 Means of parents, checks, and 191 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[c]	P1 CIR390	1.01±0.00	1.00±0.00		91.31±0.44	93.11±0.07		0.22±0.14	0.16±0.06	
	P2 B73	5.48±0.10	6.72±0.12		9.69±1.27	2.47±1.60		5.57±0.03	6.67±0.28	
	SC CG4141	4.46±0.04			13.36±1.84			4.74±0.06		
	Sabrina		5.96±0.14			9.69±3.66			6.18±0.24	
	RC KiliS4–12	1.56±0.11			62.56±3.35			2.60±0.05		
	KiliS4–8	2.40±0.07			26.45±4.53			2.73±0.07		
	F_3	2.62±0.02	2.80±0.03	2.69±0.02	47.12±1.24	46.98±1.52	47.05±0.98	3.24±5.53	3.73±0.08	3.49±0.05
Fixed effect	Location[d]			NS			NS			*
Variances[e]	$\hat{\sigma}^2_G$ F_3	1.26±0.13	2.35±0.26	1.69±0.18	540.12±57.97	781.44±85.03	647.72±69.01	1.25±0.13	1.89±0.21	1.54±0.17
	$\hat{\sigma}^2_{G\times E}$			0.09±0.02			14.12±5.49			0.04±0.02
	$\hat{\sigma}^2_{plot}$	0.02±0.01	0.17±0.03	0.08±0.01						
	$\hat{\sigma}^2_{WF}$ or $\hat{\sigma}^2_e$	0.98±0.02	1.36±0.03	1.13±0.01	44.51±5.05	84.41±9.07	62.78±4.81	0.12±0.01	0.29±0.03	0.20±0.01
$\hat{h}^2_{SL}$ design[f]		0.97	0.95	0.96	0.96	0.95	0.97	0.96	0.93	0.96

All variances $\hat{\sigma}^2_G$ were significant at 1‰; $\hat{\sigma}^2_{G\times E}$ variances were significant at 1‰ for AUT, at 1% for APIT, at 5% for ANMT; $\hat{\sigma}^2_{plot}$ were significant at 1% for AUT in Harare, at 1 ‰ for AUT in Réunion and 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-signifiant

[e] $\hat{\sigma}^2_G$, $\hat{\sigma}^2_{G\times E}$, $\hat{\sigma}^2_{WF}$; 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

QTL was putative for MSV21, MSV28, and MSV35 and for APIT. Still on chromosome 2, a putative QTL was detected for MSV07 in Harare at 47 cM. It may, or may not, be the same QTL, considering that the precision in the localization of small QTLs is very low. The QTL in bin 3.09 was detected for MSV14, MSV21, and APIT at a significant level in Réunion and at a putative level in Harare. It was significant in both environments for the later scoring dates. The QTL in bin 5.02 was detected in both environments for ANMX, and it was putative for MSV28 in Réunion, for MSV35 at both locations, and for MSV42 in Harare. The QTL in bin 6.01 was significant for MSV07 in Harare but putative in Réunion. For MSV14 and MSV21, it was significant in Réunion but putative in Harare. Even in those cases, the LR was still above 10.2, up to 18.9. For the other scoring dates, it was significant in both environments. The QTL in bin 5.03 was detected with certainty only in Harare, but there was a non-significant LR peak for the same variables in Réunion. Similarly, the QTL in bin 8.07 was detected at a significant level for ANMX in Réunion, but a non-significant LR peak existed for that variable in Harare.

◀ **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 191 segregating F_3 families from the CIRAD390×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

Most of the significant QTLs were thus detected with more certainty at the later scoring dates. Only the QTL in bin 5.03 was detected at 14 dai, and it may be involved in the early resistance. However, this needs to be confirmed as it was very small in terms of the proportion of the phenotypic variation it accounted for.

The putative QTLs were generally detected only in one environment: Harare (QTL in bins 1.10, 7.01, 9.06), or Réunion (QTL in bin 3.06, 4.02, 4.05, 9.05). The putative QTL on the long arm of chromosome 5 was detected for MSV07 and MSV42 in Réunion but at both locations for ANMX. Likewise, the putative QTL in bin 3.02 was detected for MSV07 in Harare at 26 cM. Another or most probably the same QTL was detected around 42 cM for MSV28 in Réunion and for MSV42, AUT, and APIT in Harare. We should keep in mind that the heritability at 7 dai was lower than later on; the confidence interval of the position of this minor QTL may then be quite large. However, this proves that for QTLs detected with low certainty, it is difficult to conclude if they are indeed specific for one environment or not, or specific for early or late expression of the resistance. Only the putative QTL in bin 1.10, detected for MSV07 may be preferentially involved in the early resistance as it was also detected only at this date in the DB population, which was not the case for the QTL of bin 9.06 detected for MSV07.

The significant QTLs on chromosomes 3, 5, 6, and 8 appeared to be specific for one or the other resistance component; the ones on chromosomes 5 and 8 were re-

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

Variable bin[a]	Marker interval distances	cM[b]	Place[c]	LR[d]	a[e]	d[e]	R^{2f}	Action[g]	Direction [h]	QTL×E[i]
AUT										
1.05	asg30-umc177	73	H	132.6	–1.04	–0.20	47.5	PD	PA	60.7
	62 cM-81 cM		R	122.1	–1.33	–0.53	52.7	PD	PA	
2.04	csu6a- umc8b	*65*	*H*	*10.2*	*–0.36*	*0.06*	*5.7*	*PD*	*PA*	9.5
	60 cM-66 cM		R	13.8	–0.58	0.12	8.3	PD	PA	
3.09	umc63a- umc96	140	H	15.4	0.30	–0.22	7.7	OD	PB	9.7
	139 cM-54 cM		R	17.9	0.47	–0.30	9.1	OD	PB	
6.01	umc137b- umc28	107	H	15.5	0.07	–0.52	22.5	OD	PB	10.2
	103 cM-130 cM	109	R	18.0	0.12	–0.80	29.1	OD	PB	
10.06	umc44a- bnl7.49a	36	H	43.0	–0.70	–0.35	29.6	D	PA	16.4
	35 cM-49 cM	37	R	40.3	–0.95	–0.46	30.1	D	PA	
APIT										
1.05	asg30-umc177	72	H	111.8	21.05	–0.91	43.0	A	PA	35.4
	62 cM-81 cM		R	116.8	25.74	1.88	45.4	A	PA	
3.09	umc63a- umc96	*140*	*H*	*10.0*	*–5.07*	*3.73*	*5.2*	*OD*	*PB*	7.5
	139 cM-145 cM		R	15.4	–7.70	5.32	7.8	OD	PB	
6.01	umc137b- umc28	*109*	*H*	*12.6*	*–1.74*	*9.93*	*19.4*	*OD*	*PB*	6.0
	103 cM-130 cM		R	16.7	–2.67	13.23	24.2	OD	PB	
10.06	umc44a- bnl7.49a	35	H	37.5	13.86	5.13	23.7	PD	PA	6.8
	35 cM-49 cM		R	37.5	16.39	7.41	25.6	D	PA	
ANMX										
1.05	asg30-umc177	72	H	61.1	–0.79	0.13	40.4	PD	PA	11.0
	62 cM-81 cM		R	56.4	–0.96	0.17	38.0	PD	PA	
5.02	npi409- umc90	21	H	17.0	–0.38	–0.07	9.7	PD	PA	9.0
	0 cM-21 cM		R	21.3	–0.52	–0.23	14.5	D	PA	
5.03	*umc166a- umc1*	62	H	13.8	–0.33	–0.01	6.7	A	PA	*0.5*
	56 cM-62 cM		*R*	*7.9*	*–0.33*	*0.05*	*4.3*	*PD*	*PA*	
8.07	csu38b- umc39b	104	H	*7.6*	–0.27	0.02	4.6	A	PA	12.0
	96 cM-117 cM	96	R	15.7	–0.40	0.25	11.1	OD	PA	
10.06	umc44a- bnl7.49a	36	H	33.3	–0.52	–0.22	22.9	D	PA	12.3
	35 cM-49 cM		R	38.7	–0.76	–0.18	24.5	PD	PA	

[a] Bin: estimated from the UMC1995 reference map (Coe et al. 1995)
[b] Distance: cumulative distance in centiMorgans from the first marker on the short arm of the chromosome to the position of the peak of the Lr profile
[c] Place: Harare (H), Réunion (R)
[d] Lr: Log 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
[f] R^2: percentage of the phenotypic variation explained by the QTL under consideration
[g] Gene type action, as described in Methods: A, additive; PD, partially dominant; D, dominant; OD, overdominant. Direction of the dominance is indicated by the sign of *d*
[h] Direction: origin of the allele contributing to the resistance: parent A (CIRAD390) or parent B (B73)
[i] Lr of the test of the presence of QTL×environment interactions (QTL×E)

lated to disease severity; the ones in bins 3.09 and 6.01 were related to the incidence. Note, however that in bin 3.09 a putative QTL was detected for ANMX. Some of the putative QTLs may be more related to disease severity (QTLs in bins 3.06, 7.01, 9.05, and chr5L), others to disease incidence (QTLs in bins 3.02, 4.02, and 4.05).

The percentage of the phenotypic variation (R^2) each QTL accounted for ranged from 4% to 52%, depending on the QTLs and the trait under consideration (Table 2). The major QTL in bin 1.05 explained between 16% of the phenotypic variation (MSV07 in Harare) and 52% (MSV42 in Réunion), with a mean about 45%.This QTL seemed to explain more of the phenotypic variation in Réunion than in Harare. The QTLs in bins 6.01 and 10.06 also explained quite a large proportion of the phenotypic variation: it ranged for each one from about 15% to 31%. Other significant QTLs (in bins 2.04, 3.09, 5.02, 5.03, 8.07) explained around 10% or less of the phenotypic variation. The extremes were the QTL in bin 5.02, which explained up to 15% of the phenotypic variation for ANMX in Harare, while the QTL in bin 5.03 explained only 4% for MSV14 in Harare. Most of the putative QTLs also explained around 10% of the phenotypic variation (in bins 1.10, 3.02, 3.06, 4.02, 4.05) or less (around 4% for the QTLs in bins 9.05, 9.06), except for the QTL on chr5L for MSV07 (R^2 of 35%) and the QTL in bin 7.01 for ANMX (R^2 of 20%). Generally, the proportion of the phenotypic variation explained by each QTL was somewhat more pronounced in Réunion than in Harare.

The type of gene action often involved some degree of dominance, generally in favor of the resistance (Table 2). When not, we speak of recessiveness. The QTL in bin 1.05 showed mainly a partially dominant action, except for the severity (partial recessivity). The QTL in bin 2.04 also showed a partially recessive action for MSV42 and AUT, and the QTL in bin 8.07 an overrecessive one for ANMX. The action of the QTLs in bins 3.09 and 6.01 were overdominant, whereas the action of the QTL in bin 5.02 ranged from partially dominant (in Harare) to overdominant (Réunion) for ANMX. The QTL in bin 10.05 was partially dominant or dominant. Putative QTLs showed an overdominant action, except the QTLs in bins 9.05 and 9.06 (additive action type). The QTL on chr5L had an overdominant action for MSV07 and MSV42, a dominant one for ANMX in Réunion, and an additive one for ANMX in Harare.

Origin of the resistance

The alleles brought by the parent CIRAD390 for the QTLs on chr1, chr2, two on chr5, chr8, chr9, and chr10 contributed to the resistance. For the QTL on chr6 with a small additive effect and, thus, an uncertainty on the sign of *a*, the allele contributing to the resistance likely originated from CIRAD390 as this QTL was not detected in the DB population. For the significant QTL on chr3, the allele incrementing the resistance was contributed by the susceptible parent. Kim et al. (1989) also noticed that the progeny of the resistant line IB32 crossed to B73 was, on average, more resistant than the progeny of the same resistant line crossed to another susceptible line, Mo17. For the putative QTLs, the alleles increasing the resistance were from the resistant parent (QTLs in bins 3.02, 4.02, 7.01, and chr5L), from the susceptible parent (QTLs in bins 4.05, 9.05, 9.06) or the origin remained unclear (QTLs in bins 1.10 and 3.06), because of the small value of *a*.

Stability of the QTLs in different environments

By convention, QTL × environment (QTLxE) interactions can be tested for those QTLs detected at least in one environment and by joint analysis. Consequently, these interactions were not examined for the QTLs not detected by joint analysis in bin 2.04, in bin 3.09 for MSV14 and APIT, in bin 5.03, in bin 6.01 for MSV21, MSV28, MSV35, and APIT, and for none of the putative QTLs. For the other significant QTLs, QTLxE interactions existed until MSV21 as well as for AUT, APIT, and ANMX (Table 2). For MSV28, there were no QTL×E interactions. For MSV35 and MSV42, only the region of the major QTL on chromosome 1 exhibited QTL×E interactions. This corresponds to the non-significant G×E interactions observed after 21 dai.

Importance of the QTLs

The amount (R^2) of the phenotypic variation explained by all QTLs together for each variable was similar across locations, except for MSV07. At this date, the proportion of the variation explained was higher in Réunion as for the DB population (Table 3). For MSV42, it was lower in Réunion than in Harare. As for the DB population, these percentages were similar across dates, except for MSV07, for which it was lower. The same range of the phenotypic variation was explained in both populations, from 43% to 67%. Complementary effects among QTLs probably exist because the sum of the R^2 values obtained for each individual QTL was higher than the global R^2 obtained when fitting all QTLs into the same model for a given variable.

The global gene action type of the disease score variables involved a higher degree of dominance (partially dominant to overdominant) than for the DB population, particularly in Réunion. The resistance components (incidence and severity) showed an additive action type, or

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^{2a}	a^b	d^e	l2*d*/*a* l^d	R^2	*a*	*d*	l2*d*/*a* l
MSV07	43	–0.09	–0.08	1.74	49	–0.25	–0.08	0.63
MSV14	56	–1.03	–0.33	0.63	60	–1.26	–1.56	2.49
MSV21	60	–2.05	–0.67	0.65	62	–1.92	–2.11	2.20
MSV28	63	–2.13	–1.15	1.08	62	–1.92	–2.11	2.20
MSV35	67	–2.25	–1.94	1.72	63	–2.18	–2.27	2.08
MSV42	67	–2.44	–2.26	1.85	58	–3.41	–2.75	1.61
AUT	65	–1.36	–1.29	1.89	58	–2.28	–1.97	1.73
APIT	59	34.91	4.22	0.24	61	31.77	27.84	1.75
ANMT	62	–2.24	0.27	0.24	58	–2.67	0.29	0.22
ANMX	55	–2.02	–0.16	0.16	51	–2.64	0.02	0.02

[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
[c] *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

a small degree of dominance, except for incidence in Réunion. This exception is probably a consequence of the infestation already described.

Comparison of the CIRAD390×B73 and D211×B73 populations

More QTLs were detected for the CB population than for the DB population. Between the two populations, four significant QTLs were located in the same regions of the genome (Fig. 1). The QTL in bin 1.05 was linked to the same RFLP marker, *asg30*. Both resistant parents presented the same RFLP allele. The second flanking marker in the CB population was *umc177*. This marker was not used for genotyping the DB population, but on the parental screening both parents exhibited the same RFLP allele band. Therefore, it is probable that both parents share the same resistance allele for that QTL. On chromosome 2, the presence of a QTL in the CB population confirmed the existence of at least a minor QTL in the DB population. The fact that they were not linked to the same marker may be due to the segregation distortion present in this region for the DB population. The QTL in bin 3.09 was linked to the same marker, *umc96*, for which both resistant parents had a different RFLP allele band. For the DB population, this QTL was tightly linked to *umc96* (0.2 cM). It was involved in the early resistance and its allele contributing to the resistance originated from the resistant parent. On the contrary, for the CB population, this QTL was linked only at 13.8 cM from *umc96*, was not preferentially involved in the early resistance and the allele contributing to the resistance originated from the susceptible parent. These observations are in favor of the existence of two distinct loci in this region. At the locus detected in the CB population but not in the DB population, either D211 shared the same resistance allele as B73 or epistasis exists in the DB population and masks the expression of that locus. On chromosome 10, the marker linked to the QTL presented the same RFLP allele band for both resistant parents. The fact that the QTL does not lie exactly in the same bin may be due to the imprecision of the localization. However, note that this QTL did not show the same type of gene action in both populations. Without higher precision, we are not able to say whether it is the same locus or not. Among the putative QTLs, some were also located in the same regions of the genome. The putative QTL in bin 3.02 for the CB population was detected in the same bin as a significant QTL of the DB population. This supports its existence. At the end of chromosome 1, and in chromosomes 8 and 9, putative QTLs were detected in adjacent bins. These coincidences may be considered to be an indication of the existence of these QTLs and may encourage the undertaking of intensive research on these regions.

Discussion

CIRAD390 is one of the very resistant lines to MSV, having been selected from the composite population CVR3-C3 in which different resistance genes may be present. Another completely resistant line, D211, extracted from the same composite population, was also analyzed, as described in a companion paper (Pernet et al. 1999). Information is available on lines originating from other sources (Kim et al. 1989; Kyetere et al. 1999; Welz et al. 1998). Our study was directed towards elucidating whether different genetic factors conferring resistance to MSV exist in the CIRAD germplasm, and at discovering whether they are common or related to other sources of resistance.

Control of resistance in the CIRAD germplasm

Resistance to MSV in the CB population was inherited in a quantitative manner, as in the DB population. At least eight QTLs were identified, generally acting in a dominant way. The percentage of the total phenotypic variation they accounted for varied between 43% and 67%, depending on the variable. Complementary effects between QTLs were revealed. The most important QTLs were the major one on chromosome 1 (R^2 about 45% in the CB population, up to 70% in the DB population), the QTL in bin 10.05 (25–30% in both populations), the QTL in bin 3.09 (about 25% in the DB population for the earliest scoring dates), and the QTL in bin 6.01 (about 25% in the CB population for the disease incidence). The QTL on chromosome 1 may consist of several linked genetic factors, if we refer to the profile of the LR curve (Fig. 4). This was also the case for the DB population. A consensus map cumulating data from both populations could be of some help in separating these possibly linked genes. Otherwise, larger populations should be used and marker density increased, or advanced intercrossed line populations created (Darvasi and Soller 1995).

In the present study, QTLs seemed to be stable across dates, even though some were detected with more certainty in the later resistance, when the genotypic variability between the families increased. Causes of changes in the gene action type have already been discussed elsewhere (Pernet et al. 1999). The conclusion remains valid here, namely that minor modifier genes may cause the changes. As proposed in Pernet et al. (1999), screening for MSV resistance at 14 dai, and confirmating of this scoring at 35 or 42 dai seems to be the best method for selecting resistant plants. QTLs appeared to be stable over the two environments, even though there were QTL × environment interactions for some variables.

QTL × genetic background interactions may exist, for example in bin 3.09, if the two resistant parents D211 and CIRAD390 share the same allele for the QTL detected in the CB population. Kim et al. (1989) and Pixley et al. (1997) had previously noticed the influence of one parent, the susceptible one in their case, on the progenies

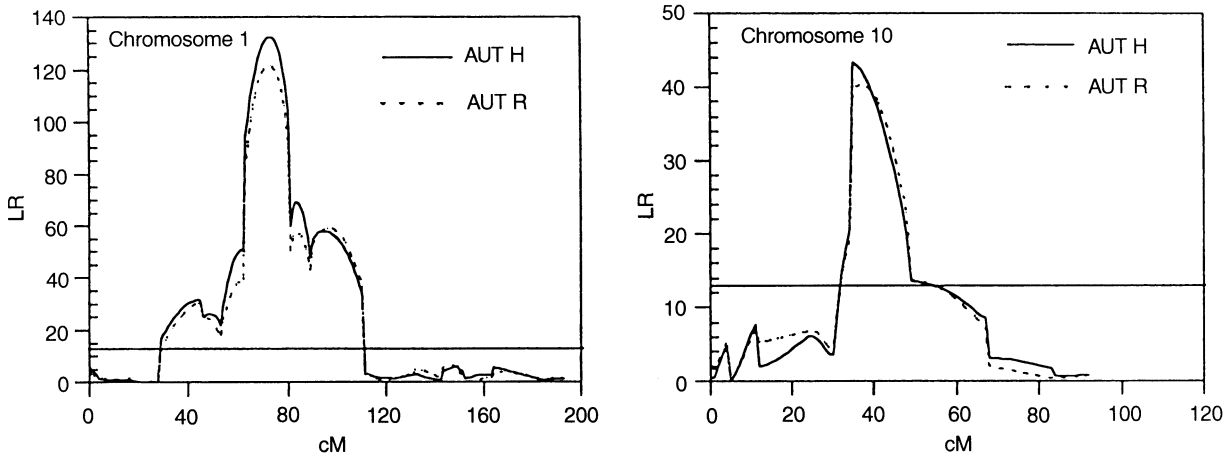


Fig. 4 QTL likelihood profiles indicating LR values for AUT in Harare (*H*) and Réunion (*R*) on chromosomes 1 and 10. The *horizontal line* indicates the level of significance at LR=13.8 (equivalent to a LOD of 3). The LR score was calculated every 1 cM

with respect to segregation for MSV resistance. This has some consequences on the selection process because some susceptible lines are particularly difficult to convert to MSV resistance (Pixley et al. 1997). In each one of these susceptible lines, some modifiers might be present that may interact negatively with the genetic factors already known. One way to study more deeply the influence of the genetic background would be to map QTLs using populations issued from factorial crosses between the donor(s) of resistance and several susceptible lines. For a transfer breeding program, it would also be particularly useful to determine if there are epistatic interactions between different regions of the genome.

As the variables APIT and ANMX are not determined by the same set of genetic factors in both populations, it is possible that two different mechanisms play a role in the resistance. The APIT variable, which integrates the number of symptom-free plants over all scoring dates, is related to disease incidence, while the ANMX variable is related to disease severity. It could be imagined that QTLs specific to the APIT variable are involved in some defense mechanisms which precociously hamper the invasion of the plant by the virus. QTLs describing the ANMX variable may be involved in some resistance mechanisms acting on the multiplication rate of the virus in the plant. This hypothesis is supported by previous studies (Peterschmitt et al. 1992; Bigarré 1994) which tend to support the fact that resistance to MSV in partially resistant lines from CIRAD is a resistance to viral multiplication. The two completely resistant lines D211 and CIRAD390 may then share with the partially resistant lines the polygenic system related to the ANMX variable and, in addition, own another polygenic system related to the APIT variable. The probable existence of several resistance mechanisms in this germplasm is favorable to the selection of a durable resistance.

Differences between genetic resistance factors present in CIRAD390 and D211 were revealed. The QTLs on chr6 and chr5 were not detected in the DB population. Some putative QTLs were detected in one population but not in the other. On chr2, differences between the two lines are also probable but this needs to be confirmed. Population sizes were not very different from each other and thus do not explain these differences. Hence, these minor QTLs were then all present in the composite population CVR3-C3, but their effects were partly masked during the selection process of the major resistance factors. They were thus randomly inherited in the final lines. The existence of different genetic factors in these two lines proves that different combinations of resistance genes from the composite population CVR3-C3 can contribute to complete resistance. Complete durable resistance may possibly be achieved through the use of various polygenic totally resistant lines.

Stability of the resistance factors across germplasm

Comparison of our results with those of previous studies enables the stability of the resistance factors to be appreciated and some consequences with respect to the selection process to be drawn.

Two genetic mapping studies have been conducted on other resistance sources. The mapping population of Kyetere et al. (1999) consisted of 87 recombinant inbred lines derived from the cross Tzi4×Hi34. The partially resistant line Tzi4 from IITA originates from the 'TZ-Y' (Tropical zea yellow) population. The other mapping population consisted of 196 $F_{2:3}$ lines obtained from the cross between CML202 (resistant) and Lo951 (susceptible). CML202 is an inbred line from CIMMYT for which the origin of the resistance remains unclear (Welz et al. 1998). The level of resistance was found to be different between the D211 and CIRAD390 Réunion lines on the one hand and the CML202 and Tzi4 lines on the other hand; D211 and CIRAD390 were rated 1, exhibiting complete resistance to MSV, CML202 was rated 2 (Welz et al. 1998), Tzi4 was rated 3 (Kyetere et al. 1999) on a whole plant 1–5 scale with half-points. All these ratings were done in Harare, the same year for the three first lines, 2 years earlier for Tzi4. Note also that none of

the four frequency distributions of MSV scores in each population were identical, which may reflect different genetic controls.

The major QTL on chromosome 1 was identified in the same marker interval for the four resistant lines. In Tzi4, this QTL, named *Msv1*, explained 94.5% of the phenotypic variation at 56 dai. It was linked to *umc167*, which lies in between the *asg30–csu92* interval of the UMC 1995 reference map. As the F_1s reaction was intermediate or similar to Tzi4, some dominance was associated with that QTL, assuming there were not any other minor genes. In CML202, this QTL explained, on average over two locations, 44% of the phenotypic variation at 21 dai, 59% at 83 dai. Its action was first determined to be additive, then partially dominant, whereas in D211 and CIRAD390 the degree of dominance decreased over time. Therefore, the four resistant lines may or may not share the same allele at that QTL. It may also be possible that different linked genes, present or not in all four resistant lines, exist at that position. From a resistance durability point of view, it is quite important to determine if the four resistance lines share the same resistance allele at that locus. Among other possibilities, near-isogenic lines with an identical genetic background could be developed and tested under different conditions or fine mapping could be done in order to test if the four resistant lines share the same marker alleles in that region.

Minor QTLs were detected in three of the four mapped populations. In the CML202×Lo951 population, three minor QTLs, explaining less than 10% of the phenotypic variation, were detected on chromosomes 2, 3, and 4 for the earlier score across the two environments at 27 dai in the CIMMYT station of Harare and at 15 dai in the Cargill station, 10 kms away and 10 days later. They were not identified for the second score, either because of a masking effect of the major QTL on chr1, or because of a decreasing effect of the minor genes (Welz et al. 1998). Note also that the precision in scoring at 87 (CIMMYT station) and 80 (Cargill station) dai is lower because many other stresses may interfere with MSV resistance. In our study, this phenomon of ”disappearance” of the minor QTLs at 35 and 42 dai was not observed, except in the case of the QTL in bin 3.09 in the DB population, which has already been discussed.

None of these minor QTLs lies in the same marker interval as those detected in the DB and CB populations. These different sets of minor QTLs can hardly be explained by differences other than genotypic. The DB and the CML202×Lo951 populations were tested in the CIMMYT station of Harare exactly at the same time. The sizes of the CB and CML202×Lo951 populations are similar, the DB population is a bit smaller. Some differences in the design (10 plants per plot for the CML202×Lo951 population as opposed to 21 plants in our case) or in the QTL analyses (use of a relatively high number, of cofactors, 12 by Welz et al.) could have led to a reduced power of QTL detection in the CML202×Lo951 population, but Welz et al. could detect minor QTLs explaining about 7% of the phenotypic variation. Welz et al. (1998) used a rating on the whole plant, which may hamper the detection of QTLs specific for a precise development stage of the disease. But even with a weekly rating on the last full expanded leaf, we detected very few QTLs of specific dates in our study. Thus, these different germplasms presumably have completely different minor factors of resistance.

No minor QTL was detected in the Tzi4×Hi34 population, either because the population was too small and the map quite sparse (82 RFLP markers) or because these minor factors were lost during the selection of Tzi4. At IITA, plants completely free of symptoms are not selected, thus taking the risk of losing factors contributing to the complete resistance. The resistance in line IB32 originating from the same resistance source was found to be inherited quantitatively with a minimum of two or three major genes according to Castle and Wright’s formula (1921) (Kim et al. 1989). As these two or three major genes were not identified in Tzi4, partial resistance of this line is most likely monogenic, or it comprises other genetic factors with very low effects. Note that the two genetic factors with very low effect mentioned in Kyetere et al. (1999) lie in regions (bins 1.11 and 9.05) where putative QTLs were identified for the CB and/or the DB populations.

The difference in the level of resistance in the four resistant lines considered may be explained either by the compound effect of the QTL on chr1 and the different minor QTLs and/or a different allele at the QTL on chr1, or different linked genes at a compound locus located at that position. Apart from this chr1-QTL, most resistance factors were shown not to be stable across germplasm. It may be possible that some of them intervene in the resistance to the insect or to the transmission by the insect, as the susceptibility was not assessed in any of the parents used in the four studies, except B73. Whatever the case, a pool of resistance genes is available for breeding varieties. As seen in the companion paper, sets of near isogenic lines containing various combinations of the detected QTLs would be also useful for studying the interactions between virus clones and genotypes, indeed even resistance factors. This will also enable us to better characterize the utility of each resistance factor and to use them according to the background and the other specific genetic factors (for example of resistance) needed in the lines to be selected.

Expanding the comparison to other diseases would enable us to check if the genetic factors contributing to the resistance to MSV are part of a cluster of resistance/tolerance genes to other tropical stresses. The minor QTL on chr3 identified by Welz et al. (1998) may be linked to the major resistance gene to maize mosaic rhabdovirus (MMV) identified in bin 3.04 by Ming et al. (1997) and Pernet et al. (1997). The QTLs in bins 3.09 (DB population) and 5.02 (CB population) occur in the same region as QTLs for resistance to Northern corn leaf blight (NCLB) (Freymark et al. 1993; Dingerdissen et al. 1996). In the region of the major QTL of chr1, resistance factors to other diseases or biotic stresses such as corn

earworm, gray leaf spot, and Stewart's wilt have been identified (review by McMullen and Simcox 1995) as well as one QTL for resistance to NCLB (Freymark et al. 1993). Still on chromosome 1, but not in the same region, a QTL for drought tolerance at flowering time has been identified (Ribaut et al. 1996). Knowledge of such linkages is of primarily importance from a selection point of view. If these resistance factors are known to be located in the same chromosomal region, marker-assisted selection could be of some help in controlling the linkage drag and facilitating their combination in a recipient line. For example, such a strategy could be employed for combining the QTLs on chromosome 1 for drought tolerance, an important abiotic stress in the sub-Saharian zone, and for MSV resistance.

The genetic mapping studies conducted by Kyetere et al. (1999) and Welz et al. (1998) and the two with Réunion lines (Pernet et al. 1999a and this paper) have increased our knowledge of the determinism of the resistance to MSV. In the populations from Réunion, the hypothesis of two resistance mechanisms was elicited. Using this new information, we have shown that completely resistant lines, with polygenic resistance, may be selected. This highly probable durable resistance will also be enhanced by the use of different genetic factors, which were shown to exist in the Mascarene germplasm and at least in the CIMMYT germplasm. Combining resistance to the insect vector with resistance to the virus could further contribute to the durability of the resistance. However, when breeders wish to decide on a selection scheme, it would be of primary interest to determine whether complete resistance is needed on a short-, mid- or long-term basis and then to combine this resistance with good yield, as MSV epidemics are erratic. The aim of combining this resistance with tolerance to other biotic (downy mildew, leaf blights, insect, Striga) or abiotic stresses, such as drought, should also be integrated in this scheme. Some studies on (1) the mechanisms of resistance to MSV, (2) the evolution of the pathogenicity of the virus, and (3) the perspectives of the maize culture in Africa will be of a great utility when determining the selection scheme.

Acknowledgments This work involved a lot of teams throughout the world. We thank D. Beck, J. Betrán, and M. Willcox from the Maize Program at CIMMYT-Mexico, for their helpful assistance in producing the material, B. Reynaud for providing the insects in Réunion. We also thank very much E. Pérez, G. Palacios, and A. Bouet for their technical assistance in the laboratory. S. Mawere, T. Gumunyui, R.-P. Hoareau, and M. Grondin are gratefully acknowledged for their help in the field work. We also thank C. Lanaud for the welcome in AGETROP lab, L. Grivet and L. Bigarré for helpful discussions. This work could not have been done without a graduate student fellowship provided by the French government. It was part of the EU contract T5–3CT920150.

References

Bigarré L (1994) Localisation *in situ* du maize streak virus (MSV) dans un maïs hybride sensible et une lignée résistante. MSc thesis, Université Paris VI, France

Castle WE, Wright SA (1921) An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. Science 54:223

Ceballos H, Deutsch JA, Guttierrez H (1991) Recurrent selection for resistance to *Exserohilum turcicum* in eight subtropical maize populations. Crop Sci 31:964–971

Clerget B, Dintinger J, Reynaud B (1996) Registration of maize inbred CIRAD390 parental line. Crop Sci 36:826

Coe E, Davis G, McMullen M, Musket T, Polacco M (1995) UMC maize RFLP and genetic working map. Maize Genet Coop Newsl 69:247–256

Darvasi A, Soller M (1995) Advanced intercross lines, an experimental population for fine genetic mapping. Genetics 141:1199–1207

Dingerdissen AL, Geiger HH, Lee M, Schechert A, Welz HG (1996) Interval mapping of genes for quantitative resistance of maize to *Setosphæria turcica*, cause of northern leaf blight, in a tropical environment. Mol Breed 2:143–156

Falconer D S (1989) Introduction to quantitative genetics, 3rd edn, Longman, London

Freymark PJ, Lee M, Woodman WL, Martinson CA (1993) Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). Theor Appl Genet 87:537–544

Henderson CR (1975) Best linear unbiased estimation and prediction under a selection model. Biometrics 31:423–447

Hoisington D, González de León D (1993) HyperMapData – a HyperCard stack for entry and analysis of molecular genetic segregation data. In: American Society of Agronomy, Crop Society of America, Soil Science Society of America (eds) Agronomy abstracts. 85th Annual Meetings. American Society of Agronomy, Madison, p 70

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Statist 6:65–70

Isnard M, Granier M, Frutos R, Reynaud B, Peterschmitt M (1997) The quasi-species nature of the maize streak virus DNA. In: ARC-Grain Crops Institute (ed) Maize Streak Dis Symp. ARC, Nelspruit, South Africa, p 11

Jiang C, Zeng Z-B (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127

John JA, Eccleston JA (1986) Row-column α-designs. Biometrika 73:301–306

Kim S-K, Efron Y, Fajesimin JM, Buddenhagen IW (1989) Mode of gene action for resistance in maize to maize streak virus. Crop Sci 29:890–894

Kyetere DT, Ming R, McMullen MD, Pratt RC, Brewbaker J, Musket T (1999) Genetic analysis of tolerance to maize streak virus in maize. Genome 42:20–26

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Linclon SE, Newburg L (1987) MAPMAKER: an interactive computer package for constucting primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Leuschner K, Buddenhagen IW, Singh J (1980) Screening for resistance to maize streak virus: an improved method of field infestation. IITA Res Briefs 1:4–6

Marchand J-L, Peterschmitt M, Reynaud B (1994) Les viroses de la striure, du stripe et de la mosaïque sur le maïs en région tropicale (Afrique et îles de l'Océan Indien). Agric Dev 4:1–16

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant-Microbe Interact 8:811–815

Ming R, Brewbaker JL, Pratt RC, Musket TA, McMullen MD (1997) Molecular mapping of a major gene conferring resistance to maize mosaic virus. Theor Appl Genet 95:271–275

Pernet A, Chavanne H, Dintinger J, Franco J, Glaszmann J-C, Hoisington D, Jewell D, Jiang C, Khairallah M, Lanaud C, Marchand J-L, Reynaud B, González de León D (1997) Genetic mapping of resistance factors to maize viruses. In: Beckstrom-Sternberg S, Shives M (eds), Plant and Animal Genome V Conf, Scherago Int., San Diego, Calif., p 102

Pernet A, Hoisington D, Franco J, Isnard M, Jewell D, Jiang C, Marchand J-L, Reynaud B, Glaszmann J-C, González de León D (1999) Genetic mapping of maize streak virus resistance from the Mascarene source. I. Resistance in line D211 and stability against different virus clones. Theor Appl Genet 99: 525–540

Peterschmitt M, Quiot JB, Reynaud B, Baudin P (1992) Detection of maize streak virus antigens overtime in different part of maize plants of a sensitive and a so-called tolerant cultivar by ELISA. Ann Appl Biol 121:641–653

Pixley KV, Jewell DC, Schechert A, Welz G (1997) Recent maize streak disease research at CIMMYT-Zimbabwe. In: ARC-Grain Crops Institute (ed) Maize Streak Dis Symp. ARC, Nelspruit, South Africa, p 4

Ribaut J-M, Hoisington DA, Deutsch JA, Jiang C, González de León D (1996) Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor Appl Genet 92:905–914

Rodier A (1995) Déterminisme génétique de la résistance du maïs (*Zea mays* L.) au maize streak virus (MSV). PhD thesis, Ecole Nationale Supérieure Agronomique de Rennes, France

Rodier A, Assié J, Marchand J-L, Hervé Y (1995) Breeding maize lines for complete and partial resistance to maize streak virus (MSV). Euphytica 81:57–70

SAS Institute (1997) SAS user's guide, version 6.12. SAS Institute, Cary, N.C.

Stuber CW, Edwards MD, Wendel JF (1987) Molecular marker-facilitated investigations of Quantitative Trait Loci in maize. II. Factors influencing yield and its component traits. Crop Sci 27:639–648

Welz HG, Schechert A, Pernet A, Pixley KV, Geiger HH (1998) A gene for resistance to the maize streak virus in the African CIMMYT maize inbred line CML202. Mol Breed 4:147–154

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468