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Breeding maize lines for complete and partial resistance to maize streak virus (MSV)

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Summary

S_1 to S_5 inbred lines, derived from a maize population bred for its overall resistance to three tropical viruses, were screened for resistance to maize streak virus (MSV) by artificial plant infection using viruliferous leafhoppers. Symptoms were rated and intra-line frequency distributions studied for all pedigree inbred lines. Mortality due to MSV was very low among these inbreds. Symptoms appeared later, developed slower and were less severe than in the susceptible control hybrid. Results of a study of 500 S_1 and 93 S_2 lines suggested that resistance is under genetic control *via* a system involving loci with major genes (with dominance for resistance) controlling high to complete resistance, associated with a genetic system involving loci with minor genes controlling partial resistance. Lines expressing complete resistance to MSV were developed from 5 cycles of inbreeding and selection. The relevance of such complete and partial resistance is discussed.

Abbreviations: MRPS – Mean Rating for Plants exhibiting Symptoms

Introduction

Streak disease of maize is caused by maize streak virus (MSV) (Bock et al., 1974), a geminivirus (Bock et al., 1977) containing circular single-stranded DNA (Mullineaux et al., 1984). The virus is exclusively transmitted by *Cicadulina* insect vectors (Storey, 1924, 1928; Webb, 1987). MSV occurs throughout Africa south of the Sahara (Bock et al., 1974; Fajemisin et al., 1984), and in the Mascarene Islands (Ricaud & Félix, 1978; Delpuech et al., 1986), many Gramineae species being hosts (Storey & Thomson, 1961). The symptoms appear as chlorotic streaks on the leaves (Storey, 1925) due to chloroplast destruction (Engelbrecht, 1982). In the worst cases, plants become completely chlorotic, leading to necrosis and death before flowering. The disease can cause serious damage to maize crops and complete yield losses have been reported (Guthrie, 1978). Widespread epidemics occurred in many African countries in 1982-83-84-86 and 87 (Malithano et al., 1987; Kim et al., 1989).

Genetic resistance was investigated and detected quite early in South Africa in the cultivar Peruvian Yellow (Fielding, 1933) and cv Arkell's Hickory. P × H, a hybrid of these two varieties, later served as an MSV-resistance donor (Rose, 1936). In P × H inbred lines, resistance was found to be mainly controlled by an incompletely dominant gene, deviations from theoretical segregation ratios being attributed to modifying genes (Storey & Howland, 1967a). In 1975, researchers at IITA (International Institute of Tropical Agriculture), Nigeria, detected resistance to MSV in cv Tropical Zea Yellow (TZY). It was then improved through mass selection and transferred to the most productive varieties (Soto et al., 1982). IB32, a TZY inbred line, was bred to S_6 in 1979. It expressed high partial resistance and is now used by IITA as an MSV-resistance donor. In IB32, resistance is con-

trolled quantitatively, mainly additively, but controlled by a relatively small number of genes (IITA, 1981; Kim et al., 1989).

Maize viruses were first reported in Réunion in 1973 and resistance was simultaneously detected in the cv Révolution (Etienne & Rat, 1973). MSV and its vector *Cicadulina mbila*, as well as maize stripe virus (MStpV) and maize mosaic virus (MMV), both transmitted by *Peregrinus maidis*, were found on the island (Delpuech et al., 1986). The population called IRAT 297 was obtained by intermating Mascarene maize populations showing resistance to the three viruses (Hainzelin & Marchand, 1986). An improved form of this population, expressing resistance to all three viruses, CVR_3-C_3 (Composite Viroses Résistant 3 – cycle 3), has been used as a resistance donor since 1986 in a programme aimed at breeding African maize varieties with resistance to MSV using backcrossing techniques (IRAT, 1986).

Our aim in the present study was to breed resistant lines and investigate the genetic control of resistance to MSV in CVR_3-C_3. This involved assessing symptom rating frequency distributions of different CVR_3-C_3 inbred lines.

Materials and methods

Plant material

In 1989, we began producing and studying resistant maize lines selfed from the CVR_3-C_3 population, at the Ligne Paradis Station, Saint Pierre, Réunion island. The S_1 generation was field-produced in winter, when MSV viral pressure is low. The following generations were also bred in the field, at different seasons by successive selfing under artificial infection. Lines were named as follows: each generation was given an abbreviated name including its descent and homozygotic stage. The 4 main S_2 lines were named with an arbitrarily chosen letter (A to D). A number was attributed to each generation (e.g. B611 represents an inbred line of the S_5 generation derived from the S_2 B line, the S_3 B6 line and the S_4 B61 line). Susceptible control hybrids were used in the different tests as follows: INRA 508 (test of the S_1 generation), INRA 518 (S_2 generation) and Sabrina (later generations). These three temperate hybrids all show complete susceptibility to MSV.

Experimental design

The S_1 generation was tested at 22 plants per inbred line with no replication. Further generations were tested at 21 plants per row in a randomized block design and three replications. A control variety was included in each block and 2 inoculated control rows were planted along the edges of each test plot. A planting density of 50 000 plants/ha was used with 80 cm row spacing and 25 cm between plants within the rows.

Infection method

The leafhoppers used for infection were mass reared (*Cicadulina mbila* population with a 100% transmission rate) (IRAT, 1986). For virus acquisition, the leafhoppers were placed for 3 days on plants, collected at the station, exhibiting very severe disease symptoms. Screening was thus carried out against a mixture of aggressive isolates. Three viruliferous leafhoppers, anaesthetized with CO_2 gas (Leuschner & Buddenhagen, 1980), were placed in the whorl of each 10-day-old test plant.

Symptom rating

MSV symptoms on plants were rated by visual evaluation on a semi-quantitative 0 to 5 scale (Table 1). S_1 lines were rated 14, 21 and 28 days postinfection, S_2 lines once a week from 7 to 42 days postinfection and S_3 to S_5 lines 28 days postinfection. This rating system was considered as the most representative.

Data analysis

For each line sample size of 21 to 63 plants the following calculations were made:

- mean symptom rating;
- mean rating for plants exhibiting symptoms (MRPS), according to the formula:

$$MRPS = \Sigma n \times N_n / \Sigma N_n$$

$1 \leq n \leq 5$: symptom rating, N_n: number of plants with an n rating, ΣN_n: number of plants expressing symptoms, MRPS = 0 when all plants are symptom-free;

- frequency (percentage) of each symptom rating (particularly the percentage of symptom-free plants), (the variables are discussed when $\Sigma N_n \leq 30$ plants) and a histogram for the frequency distribution of the

Table 1. Visual MSV symptom rating scale (adapted from Storey & Howland, 1967a)

Rate	Observed plant symptoms
0	No symptoms
1	Very slight symptoms observed by very close inspection: one or several spots or streaks on only one leaf, with no subsequent development
2	Clearly visible but limited symptoms: spots or streaks developing on several leaves, no signs (or only a few) on new leaves
3	Substantial symptoms, many long streaks, homogeneous distribution until plant maturity
4	Severe symptoms: many spread uniformly over all leaves and the whole plant; dwarfism
5	Very severe symptoms: plant completely infected, very few non-chlorotic areas, marked dwarfism, aborted reproductive organs, no progeny; or plants die of viral infection during testing

symptom ratings was plotted. Resistance levels of the S_1 parents and those of the S_2 progeny were correlated and tested by the following formula:

$$U = \nu R^2 / (1 - R^2)$$

ν = sample size - 2 = 91 R^2: square of the correlation coefficient, with U compared to an F(1; ν)-table value.

Results

Control results

In each trial, all control plants expressed symptoms within 14 days postinfection. They quickly reached the maximum symptom rating of 5, and most died before flowering.

S_1 generation results

Two sets of 250 S_1 maize lines were sown twice successively. None of the inbred lines appeared to be homogeneous (each line having several different symptom ratings). 10.8% of all individual inbreds were symptom-free. Analysis of intra-generation distributions was not possible because of the low sample size per inbred line. Among these lines, plants exhibiting different symptom ratings were selfed.

S_2 generation results

93 lines obtained by selfing S_1 plants rated 0–4 were investigated (18 to 20 lines per original rating level).

Study of all S_2 lines

The mortality rate increased from 4.3% at 14 days to 8.7% at 42 days postinfection in the CVR_3-C_3 inbred lines and from 14% to 84.2% in the susceptible check over the same period. The time-course of symptom development showed differences between the control and the CVR_3-C_3 lines relative to their parental ratings (Fig. 1).

28 days postinfection, mean symptom rating variations of the individual inbred lines were continuous, from 1.02 (inbreds of plants rated 0) to 4.9 (inbreds from parent plants rated 4). Only 6 lines showed mean ratings lower or equal to the parental rating. Standard deviations calculated for the CVR_3-C_3 lines varied from 0.4 to 1.8, the lowest values being obtained for the highest mean symptom ratings. Marked overlaps were noticed between classes concerning the mean ratings of all inbred lines from different parental ratings because of the overall high standard deviations (Table 2). The correlation coefficient between the S_1 parental symptom ratings and the mean S_2 inbred ratings was 0.55 ($p < 0.01$).

Only 10% of all inbred plants had a 0 symptom rating at 28 days postinfection. The percentage of symptom-free inbred plants increased steadily from 0 (inbred of a parent plant rated 4) to 54.5% (inbred of a parent plant rated 0). Inbreds of parent plants rated 3 and 4 all had infection rates of about 100%, except for inbred n°517 whose parental rating was 3 (Table 2). All inbred lines with more than 15% of symptom-free plants derived from parents rated 0, 1 or 2 (except for inbred n°517 which differed in all analyses, indicating that its identity should be questioned). However, there was almost 100% infection in some inbreds with the same low parental ratings. Marked overlaps were also noticed between classes concerning the percentages of symptom-free inbred plants from different parental symptom ratings (Table 2). Standard deviations were higher for inbreds from resistant plants, indicating that in these classes the reactions of S_2 inbreds sometimes showed considerable heterogeneity. This hterogeneity was lower in inbreds from susceptible parents. The correlation coefficient between S_1 parental symptom ratings and percentages of S_2 symptom-free inbreds was - 0.28 ($p < 0.01$).

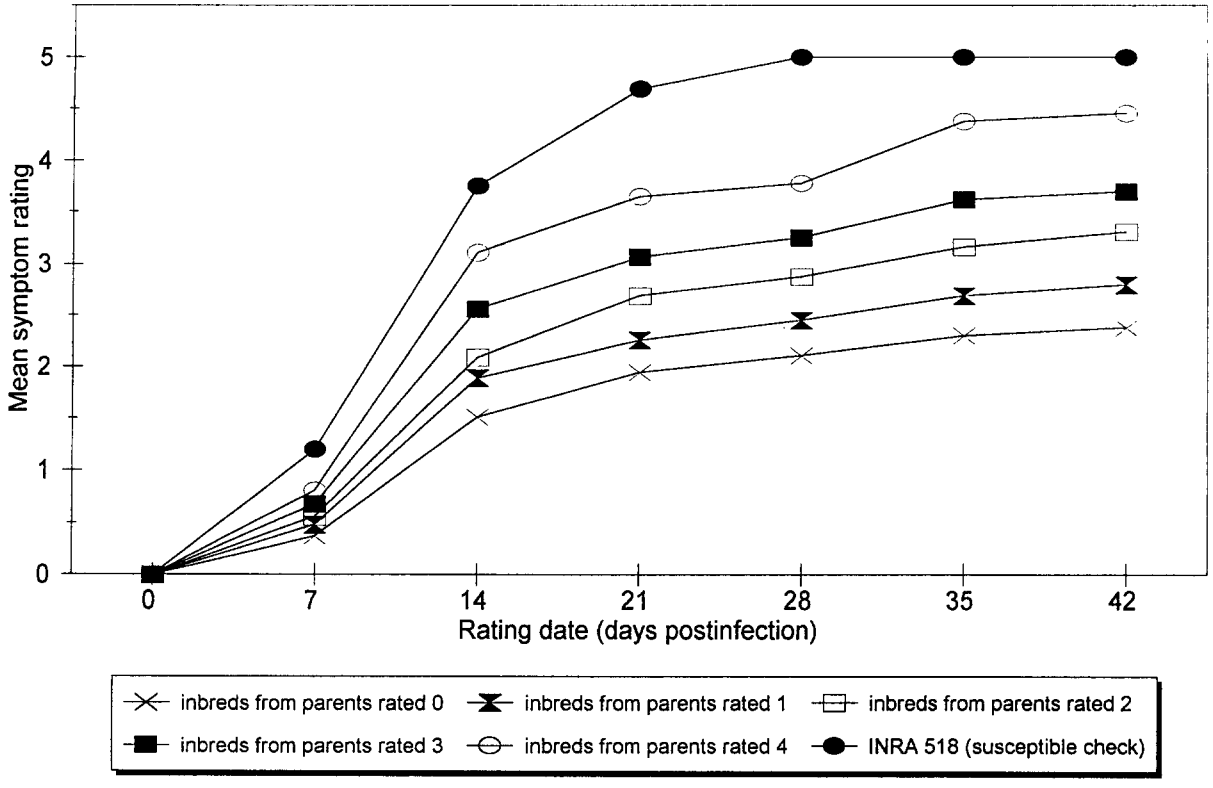

Fig. 1. MSV symptom rating in the susceptible hybrid check and S_2 lines from different parental symptom ratings (means for 18–20 inbreds from the same parental S_1 rating) at different rating dates.

Table 2. Symptom rating means and standard deviations (SD), percentages of symptom-free plants, mean ratings for plants exhibiting symptoms (MRPS) and patterns of intra-line frequency distributions observed in S_2 lines relative to their parental ratings

Parental S_1 ratings	Sample size of S_2 lines	Mean symptom rating of S_2 lines		Percentage of symptom-free plants of S_2 lines		Mean ratings for plants exhibiting symptoms (MRPS)		Number of inbreds per pattern of distribution for intra-line S_2 symptom ratings		
		Mean	SD	Mean	SD	Mean	SD	Bimodal	Unimodal	Skewed right
0	18	2.4	0.7	21.7	15.4	3.0	0.4	14	4	0
1	18	2.8	0.7	16.5	12.6	3.2	0.4	10	8	0
2	18	3.3	0.7	8.4	10.2	3.6	0.5	4	9	5
3*	18	3.8	0.7	4.3	10.3	4.0	0.5	0	7	11
4	20	4.4	0.3	1.5	2.2	4.5	0.2	0	0	20

3* except for line n°517
Rating 28 days postinfection.

The mean rating for plants exhibiting symptoms (MRPS) varied from 2.2 to 4.9. Seventy six inbreds had MRPS higher than 3 and all lines with lower levels derived from parents with symptom ratings of 0 or 1 (except for line n°101 whose parental rating was 2). All inbreds with parental ratings of 4 had MRPS higher than 4. There were also marked overlaps between classes concerning inbred MRPS from different parental symptom ratings (Table 2). The correlation coefficient

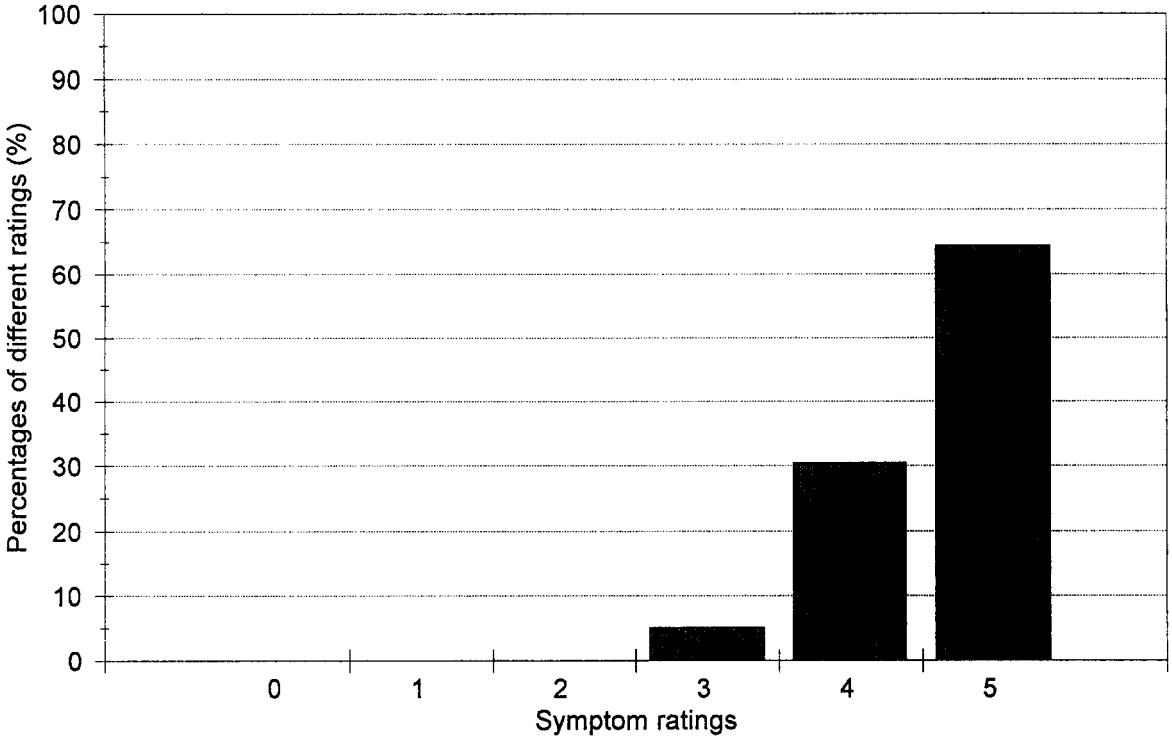

Fig. 2 a. Skewed right distribution. Inbred line from a parental rating of 4 (line n°404).

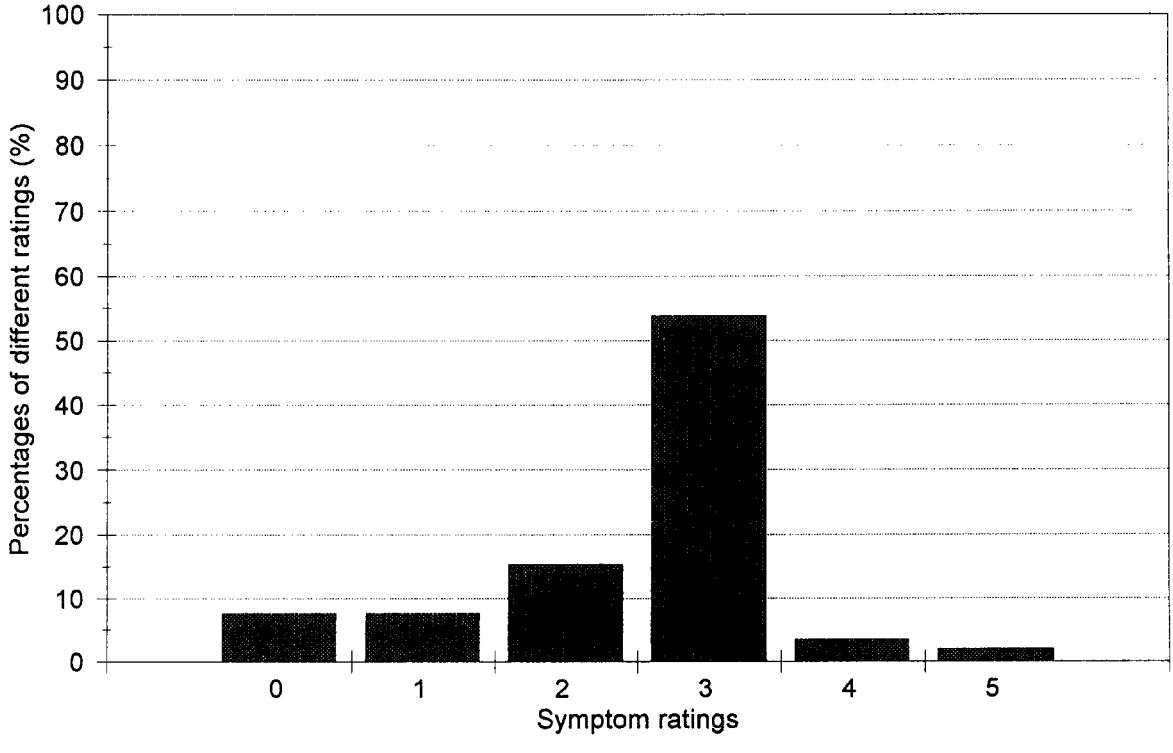

Fig. 2 b. Unimodal distribution. Inbred line from a parental rating of 0 (line n°213).

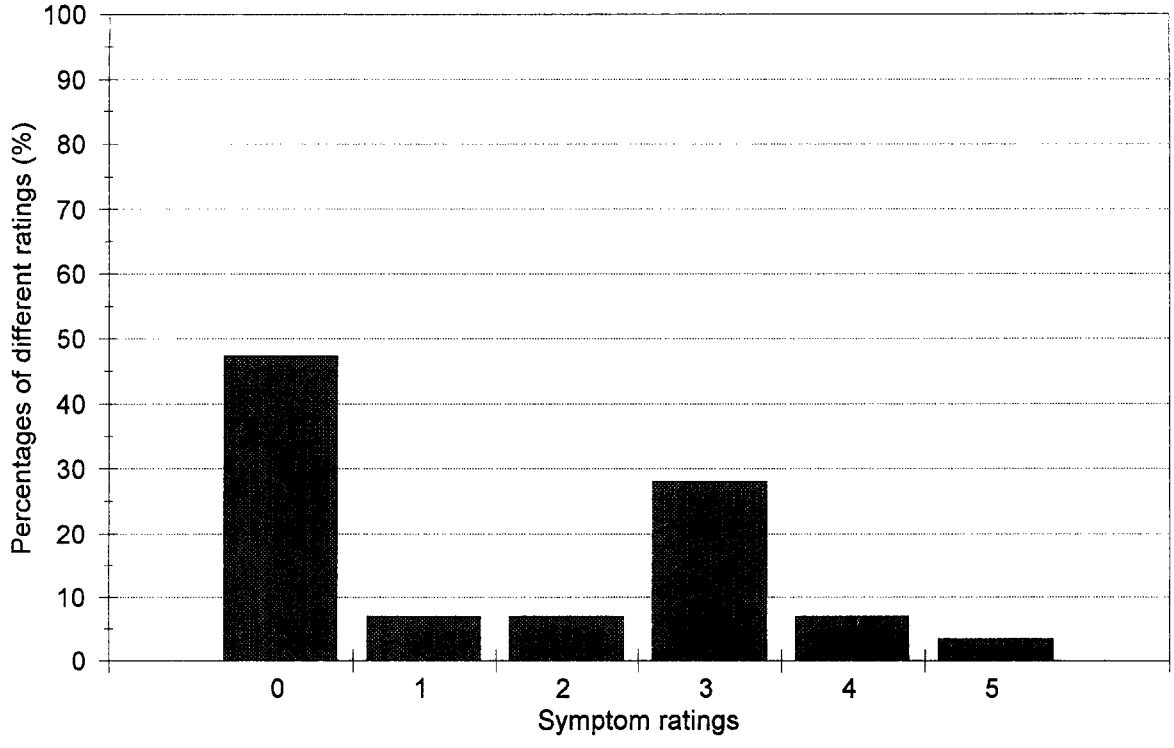

Fig. 2 c. Bimodal distribution. Inbred line from a parental rating of 0 (line B).

between the S_1 parental symptom ratings and the S_2 MRPS was 0.61 ($p < 0.01$).

Study of intra-line symptom rating distributions

Histograms for frequency distributions of the different inbred line symptom ratings revealed three distribution patterns (Fig. 2):

- skewed right distribution (Fig. 2a): very low to nil percentage of symptom-free plants, low to nil percentage of intermediate symptom ratings, very high percentage of high ratings; observed for inbreds from high parental ratings of 2, 3 and 4 (Table 2);
- unimodal distribution (Fig. 2b): low percentage of symptom-free plants, high percentage of intermediate symptom ratings, low percentage of high ratings, peak at rating 3; observed for inbreds from parental ratings of 0, 1, 2 and 3 (Table 2);
- bimodal distribution (Fig. 2c): high percentage of symptom-free plants, intermediate percentage of intermediate symptom ratings, low percentage of high ratings; observed for inbreds from low parental ratings of 0, 1 and 2, and for inbred n°517 from a parental rating of 3 (Table 2).

S_3 generation results

24 S_3 inbreds derived from 4 different resistant S_2 lines (denoted A to D), all with bimodal frequency distributions, were studied (Table 3).

Overall, no completely susceptible lines appeared, but the results were quite variable. No difference appeared between inbreds of parents rated 0 and 1. A new type of skewed left distribution was noticed: very high percentage of symptom-free plants, very low to nil percentage of intermediate symptom ratings, nil percentage of high ratings (Fig. 3).

In A inbreds, the percentages of symptom-free plants were low (1.9–12.5%), the mean ratings were from 2.1 to 2.9, and the MRPS were from 2.4 to 3. The frequency distributions were monomodal.

In five B inbreds, the percentages of symptom-free plants were high (42.1–95.8%), the mean ratings and MRPS were low (0.05–1.1 and 1–2.4 respectively). The frequency distributions were bimodal or skewed left. B5 inbred behaved quite differently: it had a very low percentage of symptom-free plants (3.5%), the mean rating and MRPS were high (2.5) and the frequency distribution was unimodal.

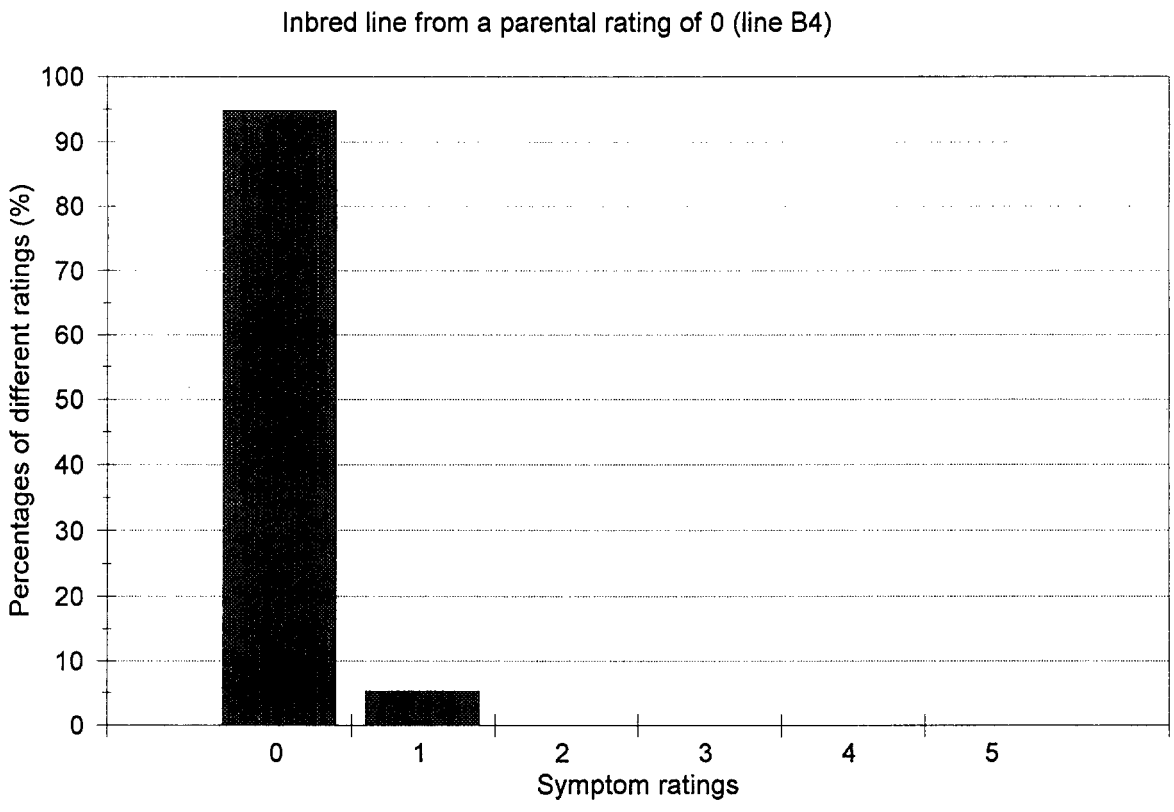

Fig. 3. Example of skewed left distribution for intra-line symptom ratings of an S_3 inbred line rated 28 days postinfection.

C inbreds showed very low to nil percentages of symptom-free plants (0–4.3%), the mean ratings and MRPS were high (2.8–3.3). The frequency distributions were generally unimodal.

D inbreds showed generally high percentages of symptom-free plants (21.8–83.8%) and the mean ratings and MRPS were low (0.2–1.8 and 1–2.3 respectively). The frequency distributions were generally bimodal. D6 had a skewed left distribution.

S_4 generation results

41 S_4 inbreds were studied (Table 4). No highly resistant inbreds could be obtained by selfing the most resistant S_3 plants of A and C lines: at the S_4 generation, the frequency distributions were unimodal, the percentages of symptom-free plants were low to extremely low (1.8–18.6%), the mean ratings and MRPS were high (2.2–2.8 and 2.8–3.1 respectively).

The percentages of symptom-free plants were lower in the C31 line than in the A lines. In B and D lines, 26 inbreds had more than 50% symptom-free plants, in 12 of these this level was above 80%. For inbreds of parent plants rated 0, percentages of symptom-free plants increased between the S_3 and S_4 generations (with 4 exceptions). From inbreds of parent plants rated 3, the percentages of symptom-free plants decreased as compared to the prior generation. The frequency distributions for these inbreds were unimodal. Unfortunately, no inbreds were produced by the D61 line which expressed complete resistance but showed poor vigour.

S_5 generation results

46 A, B and D S_5 inbred lines were studied (Table 4). The percentage of symptom-free plants was 60%. The number of lines with more than 50%, 80% and 100% of 0 symptom ratings was 29, 14 and 3 respectively.

In A lines, 5.4–51% of plants were symptom-free. The lowest percentages were obtained for inbreds from high parental ratings.

In B lines, 5.9–100% of plants were symptom-free. The lowest percentages were obtained for inbreds having parental ratings of 2 and 3, except for 2 inbreds whose parental ratings were 0. Some inbreds with parental ratings of 2 or 3 still had bimodal frequency distributions. Lines with skewed left distributions

Table 3. Percentage of symptom-free plants, mean symptom ratings and mean ratings for plants exhibiting symptoms (MRPS) of S_3 lines relative to their S_2 parents

Name of S_3 line	A1	A2	A3	A4	A5	A6	Mean
Rating of S_2 parent	0	0	1	0	0	1	
S_3 sample size	51	52	30	44	42	40	
Percentage of symptom-free S_3 plants	3.9	1.9	10.0	9.1	4.8	12.5	7.0
Mean S_3 rating	2.9	2.5	2.3	2.4	2.6	2.1	2.5
MRPS* of S_3	3.0	2.6	2.5	2.7	2.8	2.4	2.7
Name of S_3 line	B1	B2	B3	B4	B5	B6	Mean
Rating of S_2 parent	0	0	0	0	0	0	
S_3 sample size	57	53	52	57	57	48	
Percentage of symptom-free S_3 plants	42.1	50.9	61.5	94.7	3.5	95.8	58.1
Mean S_3 rating	1.1	1.2	0.9	0.05	2.5	0.08	1.0
MRPS* of S_3	1.9	2.4	2.3	1.0	2.5	2.0	2.0
Name of S_3 line	C1	C2	C3	C4	C5	C6	Mean
Rating of S_2 parent	0	0	0	0	0	1	
S_3 sample size	54	57	54	54	55	47	
Percentage of symptom-free S3 plants	0.0	0.0	1.9	1.8	1.8	4.3	1.6
Mean S_3 rating	3.1	3.3	2.8	2.8	3.0	3.0	3.0
MRPS* of S_3	3.1	3.3	2.8	2.9	3.0	3.1	3.0
Name of S_3 line	D1	D2	D3	D4	D5	D6	Mean
Rating of S_2 parent	0	0	0	0	0	0	
S_3 sample size	55	55	56	49	55	37	
Percentage of symptom-free S_3 plants	21.8	63.6	66.1	63.3	30.9	83.8	54.9
Mean S_3 rating	1.8	0.7	0.5	0.9	1.4	0.2	0.9
MRPS* of S_3	2.3	1.9	1.5	2.3	2.0	1.0	1.8

*MRPS – mean rating for plants exhibiting symptoms.
Ratings 28 days postinfection.

were all obtained from resistant plants from lines with bimodal or skewed left distributions.

In D lines, the percentages of symptom-free plants varied from 65.5% to 90.9%. This percentage increased from the S_4 to the S_5 generation except in line D342. Although D344 derived from a plant with a symptom rating of 2, its behaviour resembled that of resistant lines (90.9% of symptom-free plants).

Discussion

Expression of resistance and epidemiological effects

Infection of the susceptible check using an aggressive mixture of virus isolates was 100% successful in all tests, thus confirming the quality of infections and the validity of our screening technique.

Among all lines, 10% of S_1 and S_2 generation plants expressed no MSV symptoms. S_2 lines also had very low MSV-induced mortality and delayed appearance of symptoms, which then progressed at a slower rate and the percentage of final infection was lower than that of the susceptible check. This confirmed a definite level of resistance in our maize material.

The MSV symptom rating technique is a good means of assessing resistance since the virus is only acquired by the insect vector (Storey, 1938) and detected by serological techniques (Peterschmitt et al., 1992) in the chlorotic areas. Moreover, a correlation between viral concentration and number of symptoms has been demonstrated: selection of plants exhibiting few symptoms is thus the same as breeding for resistant plants (Peterschmitt, 1988). We use the standard terms 'complete resistance' referring to symptom-free plants in which virus multiplication is totally prevented, and 'partial resistance' referring to plants that exhibit symptoms, but to a lesser extent than the susceptible

Table 4. Filiation of S_2 to S_5 inbred lines: name, parental rating, mean symptom rating, and percentage of symptom-free plants

Name[1] of line S_2	PR[2] ind. S_1	MR[3] line S_2	%0[4] line S_2	Name[1] of line S_3	PR[2] ind. S_2	MR[3] line S_3	%0[4] line S_3	Name[1] of line S_4	PR[2] ind. S_3	MR[3] line S_4	%0[4] line S_4	Name[1] of line S_5	PR[2] ind. S_4	MR[3] line S_5	%0[4] line S_5
A	2	1.8	36.0	A2	0	2.5	1.9	A21	1	2.2	18.6	A214	2	1.3	51.0
												A215	3	2.1	20.0
												A216	3	2.7	11.7
												A217	3	2.8	5.4
												A218	2	2.2	20.8
								A22	1	2.7	9.1				
								A31	1	2.7	13.3				
B	0	1.5	47.4	B1	0	1.1	42.1	B11	0	0.1	93.5				
								B12	3	2.4	8.3				
				B2	0	1.2	50.9	B21	3	1.8	25.4				
								B22	0	0.1	91.7	B221	0	0.0	100.0
								B23	3	2.0	17.9				
								B24	0	1.3	50.8				
				B3	0	0.9	61.5	B31	1	2.7	6.9				
								B32	0	0.1	88.9				
								B33	0	1.5	50.0	B331	1	0.1	96.6
												B332	3	3.1	13.7
								B34	0	1.2	56.2	B341	0	1.5	37.3
												B342	3	1.5	47.9
												B343	1	0.9	63.5
												B344	0	0.4	76.6
												B345	0	1.7	42.6
												B346	2	2.1	33.3
								B35	0	1.7	36.0	B351	3	2.0	25.0
												B352	0	1.5	38.1
												B353	0	0.7	75.0
												B354	3	1.3	40.9
												B355	0	0.6	77.3
												B356	0	3.0	5.9
												B357	3	1.7	38.9
				B4	0	0.1	94.7	B41	1	0.0	98.3	B411	0	0.1	91.1
								B42	0	1.2	51.1	B423	2	1.4	50.0
												B424	3	2.2	21.2
												B426	0	0.9	59.6
												B427	2	0.9	64.2
				B6	0	0.1	95.8	B61	0	0.6	70.3	B611	0	0.0	98.2
												B612	2	0.6	64.8
												B613	0	1.1	54.7
												B614	2	0.8	66.7
												B615	0	0.9	56.7
												B616	2	1.7	28.6
												B617	0	0.9	64.3
												B618	0	0.0	98.3

Table 4. continued

Name[1] of line S_2	PR[2] ind. S_1	MR[3] line S_2	%0[4] line S_2	Name[1] of line S_3	PR[2] ind. S_2	MR[3] line S_3	%0[4] line S_3	Name[1] of line S_4	PR[2] ind. S_3	MR[3] line S_4	%0[4] line S_4	Name[1] of line S_5	PR[2] ind. S_4	MR[3] line S_5	%0[4] line S_5
								B62	0	0.0	98.3				
								B63	0	0.0	100.0	B631	0	0.0	100.0
								B64	0	0.0	98.4	B641	0	0.0	98.2
												B642	0	0.1	92.7
												B643	0	0.0	100.0
								B65	0	0.3	93.7	B651	0	0.4	83.3
												B652	0	0.0	98.3
												B654	0	1.2	49.0
												B655	0	0.3	84.5
C	1	1.9	32.7	C3	0	2.8	1.9	C31	1	2.8	1.8				
D	0	1.6	49.1	D1	0	1.8	21.8	D11	1	1.0	56.1				
								D12	0	1.9	35.3				
				D2	0	0.7	63.6	D21	1	0.9	63.2				
								D22	0	0.1	98.3				
								D23	1	1.2	43.5				
								D24	0	0.7	68.3				
								D25	1	1.1	50.7				
								D26	0	0.0	98.4				
				D3	0	0.5	66.1	D31	1	1.8	32.4				
								D32	0	0.3	84.7				
								D33	0	0.8	62.0				
								D34	1	0.5	75.3	D341	0	0.5	80.7
												D342	0	0.7	65.5
												D343	0	0.6	76.1
												D344	2	0.2	90.9
								D35	0	0.6	74.5				
								D36	1	1.2	52.5				
				D4	0	0.9	63.3	D41	0	2.1	23.3				
								D42	0	1.2	58.0				
				D5	0	1.4	30.9	D51	1	1.9	26.9				
								D52	1	2.3	10.0				
				D6	0	0.2	83.8	D61	0	0.0	100.0				

Symptoms ratings 28 days postinfection.
Lines with samples of 33 to 63 plants.
[1] name of line; [2] parental rating; [3] MR: mean symptom rating; [4] %0: percentage of symptom-free plants; [5] homozygous stage of the inbred line; * ind: individual.

control, and in which virus multiplication is partially suppressed (Parlevliet, 1979).

The agronomic and epidemiological benefits of complete and partial resistance include reduced yield losses and fewer inoculum sources. It was shown that the mechanism of partial resistance involves host resistance to virus multiplication. Viral particle concentrations in the leaves were found to be 10- to 90-fold higher in INRA 508 than in IRAT 297 (Peterschmitt et al., 1992).

Spreading of the disease in the field through secondary infections depends on the amount of symptoms (thus the resistance of the cultivar) and the vector population density. Once leafhoppers colonize the crop, the disease spreads in a linear way. On susceptible cultivars, infection spread becomes exponential at a vec-

tor density of one leafhopper per three plants (Rose, 1978).

Breeding resistant maize cultivars for disease control is assisted by the fact that wild Gramineae species often only have a secondary role in disease spread in croplands. Many virus isolates from these potentially alternative hosts are not able to infect and are not very aggressive on maize crops (Peterschmitt et al., 1992; Mesfin et al., 1992), indicating that cropping techniques involving weed control would not be very effective in reducing risks of viral infection.

Genetic control of resistance

In the S_1 generation, the absence of lines with specific resistance levels (particularly with 0 symptom ratings) suggests complex genetic control of resistance.

The significant correlations between parental S_1 symptom ratings and mean inbred symptom ratings, as well as percentages of symptom-free plants and MRPS of S_2 lines, indicate a connection between level of parental resistance and level of inbred lines resistance. The lowest correlation value for the percentage of symptom-free plants was due to high variation for this characteristic in inbreds from resistant parent plants (Table 2). Generally, the higher the symptom rating of the S_1 progenitor, the earlier the appearance of symptoms, the faster they progress and the more substantial they are in the S_2 inbred generation, thus confirming genetic control of resistance.

Most S_2 inbreds were more susceptible than their S_1 parents (average susceptibility gain of 1 point) and no resistant lines were derived from susceptible parent plants, suggesting that resistance was dominant. Continuous variation was noted in the mean symptom ratings, the mean rating for plants expressing symptoms and the percentage of symptom-free plants and there was no clear correlation with the parental rating. It would thus be difficult to show evidence of mendelian segregations.

Results of the data analysis, and particularly the presence of two types of frequency distributions (unimodal and bimodal) for symptom ratings of inbreds derived from resistant inbreds, seemed to indicate the existence of two different systems for genetic control of resistance in CVR_3-C_3:

- a system involving loci with major genes controlling high to complete resistance,
- a system involving loci with minor genes controlling partial resistance.

The 'major system' is supposedly monogenic or oligogenic and the breeding of lines with complete resistance seems possible. The 'minor system' is quite likely polygenic, since the frequency distributions were generally skewed around intermediate symptom ratings. In our maize material, these ratings were never fully obtained by any line.

Breeding resistant lines

Based on the possible existence of these two resistance systems, we attempted to breed maize lines with high levels of complete resistance by selfing plants showing the highest resistance (ratings of 0 and 1) from the most resistant S_2 lines (bimodal distributions). These lines are expected to have the major system capable of endoming complete resistance. The breeding process resulted in a marked improvement in resistance at the S_3 stage: no significant levels of susceptibility were noted in the 24 inbreds tested and 3 lines (B4, B6 and D6) were found to be completely MSV-resistant (skewed left distributions). Comparison of S_2 and S_3 generations revealed two different patterns:

	First pattern	Second pattern
S_2 lines names	A & C	B & D
S_1 parents ratings	2 & 1	0
S_2 lines ratings	mean rating = 1.8 & 1.9 MRPS = 2.8	mean rating = 1.5 & 1.6 MRPS = 2.9 & 3.2
S_3 lines ratings	mean rating = 2.5 & 3.0 MRPS = 2.7 & 3.0	mean rating = 1.0 & 0.9 MRPS = 2.0 & 1.8
Pattern of S_2 distribution	bimodal % 0 ratings = 36 & 32.7 % 3 ratings = 38 & 30.8	bimodal % 0 ratings = 47.4 & 49.1 % 3 ratings = 28.1 & 20.7
Predominant pattern of S_3 distribution	unimodal	bimodal and skewed left
Conclusion:	partial resistance predominant	complete resistance predominant

It seems therefore possible to breed completely MSV-resistant lines from S_2 lines showing a bimodal distribution and a high percentage of symptom-free plants (around 50%). At the S_4 generation, B and D lines had skewed left distributions and yielded inbreds showing a very high level of complete resistance. Inbreds of A and C lines were not very MSV-resistant (unimodal distributions were obtained in all cases). Selfing of susceptible plants from lines with a high percentage of symptom-free plants (bimodal) yielded inbreds with unimodal frequency distributions. It could thus be possible to breed partially resistant lines from this kind of inbreds by elimination of the major resistance

system: the ever present 'minor system' could thus be expressed. At the S_5 generation, the results confirmed our hypotheses, i.e. the A lines were not as resistant as the B and D lines. There seemed to be complete fixed resistance in some B and D lines, whereas in others resistance increased at a slower rate.

In our test conditions, the parental population CVR_3-C_3 generally produces about 10% symptom-free plants (IRAT, 1990), similar to the present results among all S_1 and S_2 lines. A high frequency of resistance was observed after 5 breeding cycles and selfing, confirming the efficiency of our screening procedure.

We compared our results with those of other studies relative to breeding of MSV-resistant lines. Storey & Howland (1967a) obtained 2 maize lines with all plants expressing resistance (including 27.6% symptom-free plants) undergoing 4 inbreeding selective cycles of 3 P × H lines with 78% resistant plants (including 6.2% symptom-free plants). Soto et al. (1982) conducted 3 selective selfing cycles with resistant TZY plants (a population with 1.9% resistant plants) and obtained lines with all plants expressing resistance. They considered that all symptom-free plants had not been infected and thus selection against a possible complete resistance may have occurred. Their lines were rated 1 or 2 (on a 0–5 scale which resembled that used in our study). The same authors bred the cv Révolution (3% resistant plants) and obtained S_1 lines with 84% resistant plants. In our conditions, similar increases in resistance were obtained after successive selfing and selection cycles but the final resistance levels were higher.

No comparisons can be made with resistance levels obtained in initial breeding programmes with P × H lines (Storey & Howland, 1967b) since this material has been lost (Bock, 1980; Soto et al., 1982). Resistance obtained in TZY lines has been successfully transferred to many hybrids and varieties (Kim et al., 1989). Some of these maize varieties, reported as being highly resistant in Africa, were found to be quite susceptible in our conditions (IRAT, 1990, 1991). Recently, highly to completely resistant hybrids have been described in South Africa (Barrow, 1992); nevertheless, these cultivars appeared to be quite susceptible in our conditions (B. Reynaud, personal communication).

Conversely, cv Révolution, originally from Réunion, was found to be resistant in Togo (Le Conte, 1974) and Nigeria (Soto et al., 1982), against the main east african MSV strains (Bock, 1980) and a south african isolate (Damstgeet, 1983). Different resistance levels have been described in this population:

- 96% resistant plants in Kenya, including 77% symptom-free plants (Bock, 1980);
- 3% resistant plants in Nigeria (Soto et al., 1982);
- 8% symptom-free plants against a south african isolate (Damstgeet, 1983);
- 5% resistant plants (none symptom-free) in Réunion (IRAT, 1991).

These variations would probably be partially due to the virus isolates used. Several different natural MSV strains, differing with respect to their virulence on maize, have been described in tests with susceptible cultivars and cv Révolution (Bock, 1980). Moreover, different isolates (from various hosts and geographical areas) were used in the above studies. In South Africa, MSV isolates from various geographical areas expressing different levels of aggressivitiy against resistant and tolerant cultivars were typed, some of them being able to overcome these plant resistances (Von Wechmar & Hugues, 1992).

Several resistant African genotypes were found to be susceptible in Réunion. In contrast, the resistance of cv Révolution has never been disproven in Africa, suggesting that MSV isolates from Réunion are more aggressive. Bock (1980) considered that MSV and its vector are endemic to this island and that the suitable climatic factors and a relative isolation of local maize populations have resulted in a steady intense selection pressure, which favoured resistance. In our conditions, some MSV isolates have been proven to be substantially less aggressive than the commonly used virus isolate mixture, but no isolates have been found that are more aggressive than the mixture (data to be published later). In our study, breeding of our resistant lines was always carried out with an aggressive virus isolate mixture.

Conclusions

Relatively few MSV-resistant maize sources are available and cv Révolution has been reported to be the best one (Damstgeet, 1983). This population is one of the 42 parents of CVR_3-C_3 (Hainzelin & Marchand, 1986). Thus, CVR_3-C_3 and our resistant lines would certainly be of interest for maize cropping throughout the MSV distribution range.

We now have several maize lines with complete fixed resistance to MSV. Some of these have been used in crossing programmes aimed at determining genetic control of resistance to maize streak virus. Sim-

ple genetic resistance systems are often overcome by the virus. Oligogenic or polygenic resistance systems could be designed which would be more difficult for the virus to overcome (Fraser, 1992).

Complete resistance could thus be used over short-term periods in creating hybrids, or over medium-term periods if backed by high partial resistance. At the same time, for long-term uses, partial resistance could be improved through standard recurrent selection, as described in studies of resistance to other plant pathogens (Parlevliet & Van Ommeren, 1988). This improved resistance might turn out to be polygenic and thus difficult for new virus isolates to overcome.

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