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Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm

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Abstract Sugarcane mosaic virus (SCMV) causes considerable damage to maize (*Zea mays* L.) in Europe. The objective of the present study was to determine the genetic basis of resistance to SCMV in European maize germplasm and to compare it with that of U.S. inbred Pa405. Three resistant European inbreds D21, D32, and FAP1360A were crossed with four susceptible inbreds F7, KW1292, D408, and D145 to produce four F_2 populations and three backcrosses to the susceptible parent. Screening for SCMV resistance in parental inbreds and segregating generations was done in two field trials as well as under greenhouse conditions. RFLP markers *umc*85, *bnl*6.29, *umc*10, *umc*44, and SSR marker *phi*075 were used in F_2 populations or F_3 lines to locate the resistance gene(s) in the maize genome. Segregation in the F_2 and backcross generations fitted to different gene models depending on the environmental conditions and the genotype of the susceptible parent. In the field tests, resistance in the three resistant European inbreds seems to be controlled by two to three genes. Under greenhouse conditions, susceptibility to SCMV in D32 appears to be governed by one dominant and one recessive gene. Allelism tests indicated the presence of a common dominant gene (denoted as *Scm*1) in all three resistant European inbreds and Pa405. Marker analyses mapped two dominant genes: *Scm*1 on chromosome 6S and *Scm*2 on chromosome 3.

Key words European maize · *Zea mays* · Sugarcane mosaic virus · Disease resistance · RFLP

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Introduction

Sugarcane mosaic is an important virus disease of maize (*Zea mays* L.) causing significant losses in grain and forage yield in susceptible genotypes (Fuchs and Grüntzig 1995). In Germany, mosaic symptoms in maize were first found in the early 1980 s near Halle (Saxony-Anhalt) in fields, where maize had been cultivated for several years (Fuchs and Kozelska 1984). Serological tests indicated that the symptoms were caused by the maize dwarf mosaic virus (MDMV) or sugarcane mosaic virus (SCMV), formerly also denoted as MDMV-A and MDMV-B, respectively (Shukla et al. 1989). Both related potyviruses are transmitted in a non-persistent manner by aphids, but efficient mechanical transmission using various artificial inoculation methods is possible. Maize dwarf mosaic is the most wide-spread virus disease of maize in the U.S. Corn Belt (Louie et al. 1991), while in Germany SCMV is more prevalent than MDMV and of increasing importance (Fuchs et al. 1996).

Diagnostic symptoms for both MDMV and SCMV include chlorosis, stunting, and reduction in plant weight (Knoke et al. 1974; Fuchs and Grüntzig 1995). The extent of damage depends on the virus strain, physiological stage of plant development at the time of infection, growing conditions of host plants, and the genotype (Louie et al. 1990). No direct chemical control for SCMV is possible. Furthermore, the control of aphid vectors through chemical means is not effective

due to non-persistent transmission of the virus. For ecological and economical reasons, the cultivation of resistant maize varieties is the most efficient method to control virus diseases.

Although a number of studies have been reported on the inheritance of resistance of U.S. maize germplasm to MDMV, the conclusions were inconsistent. Roane et al. (1977) concluded that resistance to MDMV in maize inbred Oh7B is controlled by a single dominant gene. Inbred Pa405 has shown complete resistance to MDMV and SCMV inoculation under both field and greenhouse conditions (Louie et al. 1991). Rosenkrantz and Scott (1984) reported five genes in Pa405 causing resistance to MDMV. Mikel et al. (1984) found three genes in Pa405; one gene is essential with either of the other two for complete resistance to a mixture of MDMV and SCMV. Findley et al. (1984) reported one to three major genes in Pa405 causing resistance to strains A, B, D, E, and F of MDMV. Louie et al. (1991) confirmed a single dominant gene conferring resistance to all five strains of MDMV. Recently, restriction fragment length polymorphism (RFLP) analysis mapped a major gene, *Mdm*1, near the centromere of chromosome 6 that causes resistance to MDMV in Pa405 (McMullen and Louie 1989). *Mdm*1 gene is closely linked with the nucleolus organizer region (*nor*, 0.0 cM) and RFLP marker *csu*70 (0.2 cM) in maize (Simcox et al. 1995).

Resistance of maize germplasm to SCMV and MDMV has been investigated mainly in the USA, but resistant U.S. maize germplasm such as inbred Pa405 is not adapted to the cooler climatic conditions in the maize cultivation areas of central and north-western Europe. In a recent study, Kuntze et al. (1997) screened early-maturing European maize germplasm for resistance to SCMV and MDMV and identified three dent inbreds (D21, D32, FAP1360A) but no flint inbred having complete resistance under both field and greenhouse conditions. However, information regarding the genetic basis of resistance to SCMV in early-maturing European maize germplasm is still lacking. The objectives of the study presented here to (1) determine the number of genes for resistance to SCMV in maize inbreds D21, D32, and FAP1360A, (2) compare the genetic basis of SCMV resistance of European maize inbreds with that of Pa405, and (3) identify molecular markers linked to the gene(s) involved in SCMV resistance.

Materials and methods

Plant materials

The materials involved, one resistant inbred, Pa405, from the U.S. Corn Belt, and three resistant inbreds, D21, D32, and FAP1360A, and four susceptible inbreds, KW1292, F7, D145, and D408, from the European maize germplasm. All resistant and susceptible inbreds except Pa405 have been identified in a previous study by Kuntze et al. (1997). Briefly, they screened 122 early-maturing European maize inbreds (45 flint and 77 dent lines) for resistance to SCMV and MDMV in field trials at three sites and under greenhouse conditions. Three dent inbreds, D21, D32, and FAP1360 A, were found to have complete resistance to SCMV and MDMV under both greenhouse and field conditions. Inbreds D21 and D32 are related by pedigree with coancestry coefficient $f = 0.38$. Both lines have Iodent background and one common dent parent (A632). Line FAP1360A has a largely different genetic background than D21, D32, and Pa405 except for some distant common sources, including line Co125 and other dent inbreds from Canada and Wisconsin. Likewise, Pa405 has a different genetic background than the resistant European inbreds. The inbreds KW1292, F7, D145, and D408 showed a high degree of susceptibility to SCMV and were unrelated except for F7 and D145, whose ancestor line F_2 originated also from population 'Lacaune' as did F7. Detailed pedigrees of the inbreds can be obtained upon request from the corresponding author.

Inoculum preparation and inoculation

Virus inoculum for testing resistance against SCMV isolate "Seehausen" was prepared as described by Fuchs and Grüntzig (1995). Young leaves with typical mosaic symptoms of the SCMV-infected maize variety 'Bermasil' were homogenized using 5 volumes of a 0.01 *M* phosphate buffer at pH 7.0. Carborund was added to the sap. During sap preparation and mechanical inoculation, the inoculum was kept at $+4^{\circ}$C. Plants at the three- to four-leaf stage were mechanically inoculated twice at a weekly interval by an air brush technique with a tractor-mounted air compressor at a constant pressure of 800 kPa (Fuchs et al. 1996).

Test of allelism

The three resistant European maize inbreds D21, D32, and FAP1360 A were crossed with U.S. resistant inbred Pa405 for performing an allelism test. The experiment was conducted in the greenhouse from May to June in 1995. Plants of the F_1 and F_2 generations of each cross were planted in pots with five seeds per pot. Fifty-five F_1 plants of cross D21 × Pa405, 9 F_1 plants of cross D32 × Pa405, and 14 F_1 plants of cross FAP1360 A × Pa405 were tested for resistance to SCMV. In addition, 200 F_2 plants of each cross were screened for SCMV resistance. Resistance was evaluated by scoring each plant for the presence or absence of mosaic symptoms. Mosaic symptoms were rated at weekly intervals. Final rating of mosaic symptoms was performed 35 days after the initial inoculation. In addition, all plants were examined serologically 35 days after initial inoculation using a double antibody sandwich (DAS) ELISA according to Flegg and Clark (1984). Optical density was measured photometrically at a wavelength of 405 nm (Reader, Bio-Thek Instrument).

In addition, resistant inbred D21 was crossed with FAP1360 A for performing a test of allelism among European lines under field conditions. About 250 F_2 plants and their parental inbreds were tested for resistance to SCMV in a field trial at Hohenheim, near Stuttgart, Germany, in 1997. Seeds were planted in single-row plots 0.75 m apart and 4 m long. Mosaic symptoms were rated at weekly intervals, with the final rating performed 47 days after initial inoculation.

Segregation analysis

Three resistant European inbreds were crossed with four susceptible inbreds (F7, KW1292, D408, D145) to produce four F_2 and three backcross progenies to susceptible parents (Table 1). For each cross,

Table 1 Total number of plants and percentage of infected plants in parental inbreds and segregating generations of different crosses in European maize as well as checks evaluated in field and greenhouse trials recorded 46–49 days after initial inoculation (DAI) with SCMV

	Hohenheim		Eckartsweier		Greenhouse	
	Total	46 DAI	Total	47 DAI	Total	49 DAI
		(%)		(%)		(%)
F7	74	100	82	82	20	100
FAP1360A	87	0	90	0	21	0
F7 × FAP1360A F_1	85	0	81	0	49	67
F7 × FAP1360A F_2	418	24	410	18	103	76
(F7 × FAP1360A) × F7	425	58	417	22		
KW1292	76	100	80	89	25	100
FAP1360A	84	0	73	0	18	0
KW1292 × FAP1360A F_1	42	0	46	0	50	2
KW1292 × FAP1360A F_2	405	20	378	13	139	26
(KW1292 × FAP1360A) × KW1292	412	40	395	25		
D408	72	100	79	83	47	100
D21	69	0	82	0	35	0
D408 × D21 F_1	41	2	81	0	–[c]	–
(D408 × D21) F_2	377	22	385	15	140	23
(D408 × D21) × D408	369	53	403	28		
D145	42	100	41	78	37	100
D32	40	0	31	0	40	0
D145 × D32 F_1	37	5	41	0	20	85
D145 × D32 F_2	415	99	404	13	175	78
Pa405[a]	83	0	6	0		
F7[b]	522	99	566	73		

[a] Resistant check
[b] Susceptible check, values are averaged
[c] Not tested

about 400 F_2 and 400 BC_1 plants were screened for SCMV resistance in two field trials each conducted at Hohenheim and Eckartsweier, near Offenburg, Germany, in 1995. Each subexperiment also included a minimum of 40 plants of each parental inbred and 40 F_1 plants of each cross. In addition, 80 plants of the susceptible check F7 and 20 plants of the resistant check Pa405 were also tested to confirm that the inoculation technique was effective. Seeds were planted in single-row plots 0.75 m apart and 4 m long. All plants were inoculated twice with SCMV. When the susceptible check showed the first mosaic symptoms, all plants were evaluated for the presence or absence of mosaic symptoms at 4-day intervals during the first 3 weeks after initial inoculation. Subsequent ratings were performed at weekly intervals. Final ratings for mosaic symptoms were taken 46 days after initial inoculation at Hohenheim and 47 days after initial inoculation at Eckartsweier.

In a further experiment, 200 F_2 individuals of each cross were tested for SCMV resistance under greenhouse conditions. F_2 populations of crosses F7 × FAP1360 A and KW1292 × FAP1360 A were screened during February and March in 1995, while those of D408 × D21 and D145 × D32 were tested during October and November in 1995. In each trial, a certain number (Table 1) of plants of the F_1 generation and parental inbreds were included as multiple entries. F_2 plants of each cross were planted in 40 pots with five seeds per pot. Parental inbred lines and the F_1 generation of each cross were also planted with five seeds per pot. Ratings for mosaic symptoms due to SCMV were performed at weekly intervals. Final rating was done 49 days after initial inoculation.

Tissue print immunoblotting test

A tissue print immunoblotting (TPIB) test described by Hohmann et al. (1996) was performed to confirm visual scoring. All plants of the field experiments, including parental inbreds, F_1, F_2, and backcross populations, as well as susceptible and resistant checks, were tested 4 weeks after initial inoculation. Symptomless plants of the field experiments were evaluated 7 weeks after initial inoculation. All plants of the greenhouse experiments were tested by TPIB after final rating. Leaves were cut with scissors and labeled individually according to plant number. Each leaf was subsequently cut with a new razor blade to obtain a plane-cut surface. Tissue blots were obtained by pressing the freshly cut leaf surface onto a 0.45-μm pore size nitrocellulose membrane (Boehringer, Mannheim). The immunological detection of antigens on tissue blots was performed by the protocol of Hohmann et al. (1996).

Statistical analysis

The rate of infection was calculated for each environment using plants of susceptible parental inbreds after final rating. In case the infection rate of the susceptible parent was less than 100%, the number of infected plants in F_2 and backcross progenies was arithmetically corrected using the follwing correction formula: $X = X^* \times 100 / I$, where X is number of infected plants after correction, X* is the actual number of infected plants, and I is the rate of infection in the susceptible parent. For various genetic hypotheses, χ^2 tests for the goodness of fit between observed and expected numbers of diseased and symptomless plants were performed according to the standard procedure described by Weir (1990).

RFLP and SSR analyses

From four F_2 populations tested in the field trial at Hohenheim in 1995, leaf material was collected from 10–20 susceptible and 10

symptomless plants (see Table 3). Genomic DNA was extracted from leaf material and digested with restriction enzymes *Eco*RI, *Eco*RV, *Hin*dIII, and *Bam*HI. The resulting DNA fragments were separated by agarose gel electrophoresis and transferred onto uncharged membranes by Southern blotting. Hybridization was performed with genomic DNA probes *umc*85 and *bnl*6.29 from the standard probe collection available at the University of Missouri, Columbia (Gardiner et al. 1993). These RFLP markers were chosen because they flank *Mdm*1 (estimated map distance of 0.5 cM and 0.4 cM, respectively), a gene known to confer resistance to MDMV in the U.S. inbred Pa405 (McMullen and Louie 1989; Simcox et al. 1995). Probes were labeled with digoxigenin-dUTP, and DNA fragments were detected by means of the chemiluminescence CSPD protocol described by Hoisington et al. (1994). Probe *umc*85 did not detect polymorphism between D145 and D32 for any of the four restriction enzymes.

Genomic DNA of F_2 plants from cross D145 × D32 was additionally used for microsatellite analysis with simple sequence repeat (SSR) marker *phi*075 following the protocol of Senior et al. (1996). Primer sequences for *phi*075 were obtained from the maize database (http://teosinte.agron.missouri.edu/Coop/SSR_Probes/SSR1.htm). According to Senior et al. (1996), *phi*075 maps to chromosome 6 in the vicinity (10–20 cM) of the *Mdm*1 gene.

For all crosses except D408 × D21, F_2 plants included in the RFLP analyses were selfed to produce F_3 lines. These F_3 lines were tested for resistance to SCMV in the greenhouse in the winter of 1996. Each F_3 line was planted in 4 pots with five seeds per pot. The experiment also included 20 plants of each parental inbred line. All plants were inoculated with SCMV. Mosaic symptoms were rated at weekly intervals. Final rating was performed 47 days after the initial inoculation. In addition, all plants were tested by TPIB after the final rating, as described above. Two F_3 families derived from F_2 plants with number 146 in cross F7 × FAP1360 A and 280 in cross D145 × D32 were homozygous for the RFLP band of the resistant parent at markers *umc*85 and *bnl*6.29, respectively, flanking the *Mdm*1 gene. However, they exhibited both resistant and susceptible plants in the first greenhouse experiment. These two F_3 families were tested a second time under greenhouse conditions in 1996. Each F_3 line was planted in 14 pots with five seeds per pot. Parental inbreds were included with 20 plants per line. Final rating was performed 47 days after initial inoculation. All plants were tested by TPIB. Leaf samples were collected from 20 susceptible and all resistant plants of each F_3 line (#146, #280) for subsequent RFLP assays. Southern hybridization was performed with three RFLP probes, *bnl*6.29 (chromosome 6), *umc*10 (chromosome 3), and *umc*44 (chromosome 10) linked to genomic regions known to confer resistance in Pa405 to wheat streak mosaic virus (WSMV), a potyvirus closely related to MDMV (McMullen et al. 1994).

The *G*-test of independence described by Sokal and Rohlf (1981, p. 745) was used in F_2 populations and F_3 families of different crosses to test the goodness of fit of the observed frequencies of marker genotypes at various RFLP markers and SSR marker *phi*075 with their expected frequencies under independent segregation from the resistance gene(s). Williams' correction factor was used to adjust for the Type-I error in the *G*-test. The *G* value was compared with the critical value of χ^2 at 2 degrees of freedom.

Results

Visual ratings and TPIB analysis for mosaic symptoms due to SCMV produced identical results in 98% of the plants across different crosses (data not shown). In the field experiment at Hohenheim in 1995, 2–6 plants (out of >400 plants) in the F_2 and backcross generation of cross F7 × FAP1360A and cross KW1292 × FAP1360A produced contrasting results in visual scoring and TPIB analysis, i.e., plants rated as susceptible by visual scoring showed resistant reaction in TPIB analysis or vice versa. Out of about 400 plants each in F_2 and backcross generation, 2–18 plants in crosses D408 × D21, (D408 × D21) × D408, and D145 × D32 at Hohenheim and 4–13 plants across different crosses at Eckartsweier were scored as resistant by visual observations but declared susceptible in TPIB analysis.

Screening for resistance to SCMV

All plants of D21, D32, and FAP1360A as well as resistant check Pa405 were without any SCMV symptoms in the field and greenhouse tests (Table 1). Infection rate in susceptible inbreds was 100% at final rating in the greenhouse and field trials at Hohenheim, while it ranged from 78% to 89% in field trials at Eckartsweier. Accordingly, the number of infected plants in the F_2 and BC_1 generations were corrected corresponding to the infection rate of the susceptible parent in a particular cross for performing χ^2 tests using the described formula. In the field trials, the infection rate of the susceptible check F7 at the final rating averaged 99% at Hohenheim and 73% at Eckartsweier.

All F_1 plants of crosses F7 × FAP1360 A and KW1292 × FAP1360 A were resistant in the field trial at Hohenheim in 1995 (Table 1). In contrast, crosses D408 × D21 and D145 × D32 displayed 2% and 5% susceptible plants in the F_1 generation, respectively. F_1 plants of all crosses were resistant in the field trial at Eckartsweier. Under greenhouse conditions, the proportion of SCMV-infected plants in the F_1 generation was 67% in cross F7 × FAP1360 A, 85% in cross D145 × D32, and just 2% in cross KW1292 × FAP1360 A.

Inheritance of resistance to SCMV

Single-gene model

In all crosses between resistant and susceptible European inbreds, χ^2 tests for the goodness of fit for a single-gene model were performed with the data obtained at the final ratings (Table 2). In cross D408 × D21, a good fit of observed with expected segregation ratios, 3r : 1s, under a single dominant gene model was found for the F_2 generation in the field test at Hohenheim and under greenhouse conditions. Segregation in the F_2 generation of cross F7 × FAP1360A also fitted a 3r : 1s ratio in both field trials but not in the greenhouse test. Conversely, in the F_2 generation of cross KW1292 × FAP1360A, significant deviations from a single dominant gene model were observed in the field trials but not in the greenhouse test. Likewise, significant deviations from a single gene model were observed

Table 2 Test of different gene models for segregation of SCMV resistance in F_2 and backcross generations (BC) of different croses in European maize evaluated in field trials at Hohenheim (HOH) and Eckartsweier (EWE) as well as under greenhouse (GH) conditions

Cross (S × R)[a]	Trial	F_2						BC (S × R) × S					
		Resistant	Susceptible	Expected ratio[b]	χ^2	Expected ratio[c]	χ^2	Resistant	Susceptible	Expected ratio[b]	χ^2	Expected ratio[b]	χ^2
		Number of plants		One gene		Multiple genes		Number of plants		One gene		Multiple genes	
KW1292 × FAP1360A	HOH	332	83	3r : 1s	4.4*	13r : 3s	0.78	245	167	1r : 1s	14.6**	1r : 1s[d]	14.6**
	EWE	323	55	3r : 1s	21.5**	54r : 10s	0.32	284	111	1r : 1s	75.6**	3r : 1s	1.94
	GH	103	36	3r : 1s	0.05	13r : 3s	4.7*						–
D408 × D21	HOH	294	83	3r : 1s	1.80	13r : 3s	2.5	174	195	1r : 1s	1.19	1r : 1s[d]	1.19
	EWE	315	70	3r : 1s	9.5**	54r : 10s	0.06	267	136	1r : 1s	42.6**	3r : 1s	16.1**
	GH	108	32	3r : 1s	0.36	13r : 3s	1.54						–
F7 × FAP1360A	HOH	317	101	3r : 1s	0.16	13r : 3s	8.3**	180	245	1r : 1s	9.9**	1r : 1s[c]	9.9**
	EWE	320	90	3r : 1s	2.02	54r : 10s	12.5**	305	112	1r : 1s	89.2**	3r : 1s	0.81
	GH	79	147	1r : 3s	11.4**	11s : 5r	1.31						
D145 × D32	HOH	231	184	3r : 1s	82.8**	9r : 7s	0.06						
	EWE	337	67	3r : 1s	15.2**	54r : 10s	1.04						
	GH	40	135	1r : 3s	0.43	13r : 3r	1.8						

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

[a] Susceptible × resistant

[b] Expected ratio corresponds to a one-gene model

[c] Expected ratios 13 : 3, 11 : 5, and 9 : 7 correspond to two-gene models (Strickberger 1985), and ratio 54 : 10 corresponds to a three-gene model: 13r : 3s, one dominant and one recessive gene acting independently for resistance; 13s : 3r, one dominant and one ressive gene acting independently for susceptibility; 9r : 7s, two complementary dominant genes; 11s : 5r, two dominant genes, one must be in homozygous condition for resistance; and 54r : 10s, three genes, any two in dominant condition produce resistance

[d] 1 : 1 ratio corresponds to two-gene model

Table 3 Segregation ratios for different molecular markers flanking presumed gene(s) for resistance to SCMV in F_2 populations and F_3 lines of different crosses in European maize

Cross (P1 × P2)[a]	RFLP marker	Phenotype for SCMV resistance	Observed number of plants with RFLP bands			*G* value
			P1P1	P1P2	P2P2	
KW1292 × FAP1360A F_2	*umc*85	Resistant	0	7	3	
		Susceptible	10	0	0	24.5**
	*bnl*6.29	Resistant	0	6	4	
		Susceptible	10	0	0	24.8**
D408 × D21 F_2	*umc*85	Resistant	1	4	5	
		Susceptible	20	0	0	28.2**
	*bnl*6.29	Resistant	0	5	5	
		Susceptible	20	0	0	35.7**
F7 × FAP1360A F_2	*umc*85	Resistant	0	8	2	
		Susceptible	13	5	0	16.4**
D145 × D32 F_2	*bnl*6.29	Resistant	0	8	2	
		Susceptible	6	11	2	5.3
	*phi*075[b]	Resistant	0	5	4	
		Susceptible	6	12	1	8.9*
F7 × FAP1360A F_3 (line #146)	*umc*10	Resistant	0	2	11	
		Susceptible	1	5	1	8.0*
	*umc*44	Resistant	2	9	2	
		Susceptible	0	2	5	5.9
D145 × D32 F_3 (line #280)	*umc*10	Resistant	0	3	4	
		Susceptible	5	6	2	6.2*
	*umc*44	Resistant	4	3	0	
		Susceptible	6	6	1	0.7

*,** Significant at the 0.05 and 0.01 probability levels, respectively
[a] Susceptible (P1) × resistant (P2)
[b] Data from microsatellite (SSR) analysis

in the F_2 generation of cross D145 × D32 in two field tests, while under greenhouse conditions, segregation was consistent with a single recessive gene model. Significant deviations from a single-gene model were observed in the backcross generation of all crosses in the field tests except for (D408 × D21) × D408 at Hohenheim.

Multigene models

In the F_2 generations of all four crosses except F7 × FAP1360 A, two-gene models (segregation ratio 13r : 3s or 9r : 7s) or a three-gene model (54r : 10s) yielded a better fit with observed results in field trials at Hohenheim and Eckartsweier, respectively, than the one-gene model (Table 2). This applied also to the results of the BC generation from the field trials at Eckartsweier. However, no improvement was obtained with more complex models for the F_2 populations tested in the greenhouse except for the cross F7 × FAP1360A, in which segregation was consistent with a 11s : 5r ratio of a two-gene model.

Molecular marker analyses

In cross KW1292 × FAP1360A, the 10 resistant F_2 plants were either homozygous or heterozygous for the RFLP band of the resistant parent FAP1360A (P2) at markers *umc*85 and *bnl*6.29 (Table 3). Conversely, all 10 susceptible F_2 plants were homozygous for the RFLP band of the susceptible parent KW1292 (P1). The *G*-test for independent segregation of marker genotypes in the resistant and susceptible class was highly significant ($P < 0.01$) for *umc*85 and *bnl*6.29, indicating the presence of a resistance gene to SCMV on the short arm of chromosome 6 in the resistant inbred FAP1360A. All 20 F_2 plants had identical marker genotypes at both marker loci except for 1 resistant plant that was homozygous P2P2 for *bnl*6.29 but heterozygous for *umc*85 (Table 3). In the greenhouse test, all F_3 lines derived from the 10 susceptible F_2 plants were completely susceptible. F_3 lines derived from the 10 resistant F_2 plants were completely resistant only in those three cases in which the parental F_2 plant was homozygous P2P2 for both markers, but otherwise they segregated into susceptible and resistant plants. Similar results as for this cross were observed at RFLP markers *umc*85 and *bnl*6.29 for the F_2 generation of cross D408 × D21 except that in the class of resistant plants, 1 plant was homozygous for the marker genotype P1P1 of the susceptible parent D408 at *umc*85 (Table 3).

In cross F7 × FAP1360A, resistant F_2 plants were either homozygous or heterozygous for the RFLP band of the resistant parent FAP1360 A (P2) at marker

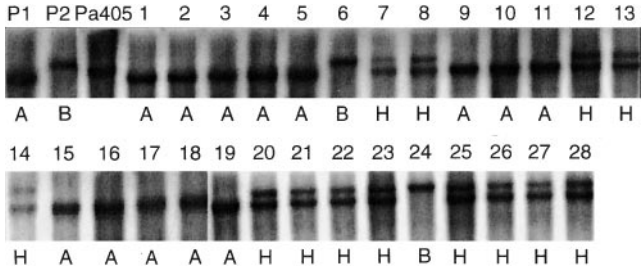

Fig. 1 RFLP banding patterns of marker *umc*85 in susceptible parental inbred F7 (P1), resistant parental inbred FAP1360A (P2), resistant inbred Pa405, and 18 susceptible (*lanes 1–5, 7–19*) and 10 resistant (*lanes 6, 20–28*) F_2 plants from cross F7 × FAP1360A. *A* is homozygous for the RFLP band of susceptible parent, *B* is homozygous for the RFLP band of the resistant parent, and *H* is heterozygous

*umc*85 and, conversely, susceptible F_2 plants were either homozygous or heterozygous for the RFLP band of the susceptible parent F7 (P1) (Table 3). The *G*-test was highly significant ($P < 0.01$), indicating the presence of a SCMV resistance gene on chromosome 6S. RFLP banding patterns of *bnl*6.29 could not be scored in this cross due to multiple overlapping bands. In greenhouse tests in 1996, F_3 lines developed from the 13 susceptible F_2 plants homozygous P1P1 at marker *umc*85 showed SCMV symptoms. Two of the five F_3 progenies derived from 5 susceptible F_2 plants heterozygous at marker *umc*85 segregated for SCMV resistance, whereas the other three F_3 lines were completely susceptible. Likewise, six of the eight F_3 lines derived from 8 resistant F_2 plants heterozygous at RFLP marker *umc*85 segregated into resistant and susceptible plants, while two were completely susceptible. The F_3 line of 1 resistant F_2 plant homozygous for the RFLP band of the resistant parent FAP1360A at marker *umc*85 was completely resistant. Conversely, the F_3 line developed from 1 resistant F_2 plant (#146, lane 24 in Fig. 1) homozygous for the RFLP band of the resistant parent FAP1360 A at markers *umc*85 segregated into 13 resistant and 46 susceptible plants.

In cross D145 × D32, none of the resistant F_2 plants was homozygous for the marker genotype of the susceptible parent D145 (P1) at marker loci *bnl*6.29 and *phi*075, but all three marker genotypes occurred in the susceptible class of F_2 plants (Table 3). The *G*-test for independent marker genotype segregation in the resistant and susceptible class of F_2 plants was significant ($P < 0.05$) only for *phi*075 but barely below the critical threshold ($P = 0.07$) for *bnl*6.29, indicating the presence of a resistance gene on chromosome 6S in agreement with the other crosses. The F_3 line derived from 1 resistant F_2 plant (#280) homozygous for the RFLP band of the resistant parent D32 at marker *bnl*6.29 segregated into 7 resistant and 63 susceptible plants under greenhouse conditions in 1996.

When the two F_3 lines (#146 of cross F7 × FAP1360 A and #280 of cross D145 × D32) were further examined by RFLP analysis, the results confirmed that all plants were homozygous for the RFLP band of the resistant parent at markers *umc*85 and *bnl*6.29 (data not shown). All resistant plants in each F_3 line were either heterozygous or homozygous for the RFLP band of the resistant parent at marker *umc*10 (Fig. 2, Table 3). In contrast, all three kinds of marker genotypes at *umc*10 occurred among susceptible F_3 individuals in both lines. The *G*-test confirmed linkage of a resistance gene with RFLP marker *umc*10 on chromosome 3 in each F_3 line of crosses F7 × FAP1360 A and D145 × D32. Segregation ratios of marker genotypes at marker *umc*44 did not indicate the presence of any resistance gene in this region of chromosome 10 (Table 3).

Test of allelism

Both F_1 and F_2 populations of the three crosses of resistant European inbreds with U.S. inbred Pa405 did not show any segregation for susceptible plants under greenhouse conditions in 1995. Likewise, all 247 F_2 plants in cross D21 × FAP1360 A were completely resistant under field conditions at Hohenheim in 1997. Results were confirmed by ELISA, which showed complete agreement with the visual scoring.

Discussion

Environmental instability of resistance

Large differences were observed between two field environments and greenhouse conditions with respect to the proportion of susceptible plants in susceptible parental inbreds and segregating generations of various crosses (Table 1). A direct relationship between susceptibility of maize to MDMV with increasing temperature was reported by Tu and Ford (1969). Conversely, the comparison of two field experiments in our study demonstrated that in spite of a higher temperature at the time of rating mosaic symptoms, the proportion of susceptible plants was lower at Eckartsweier than at Hohenheim. At Eckartsweier, the average temperature was 16.7°C in June and 21.9°C in July as compared to 14.8°C and 21.1°C, respectively, at Hohenheim.

The F_1 and F_2 generation of two crosses, F7 × FAP1360 A and D145 × D32, produced a greater proportion of susceptible plants under greenhouse conditions than field tests. Louie et al. (1990) showed that the average incidence of MDMV in ten inbreds in the greenhouse was 50%, while the same inbreds averaged 4% in the field. Furthermore, the disease incidence of four inbreds changed from nearly 100% in the greenhouse to nearly 0% in the field. They hypothesized that plants in the greenhouse are more tender and that

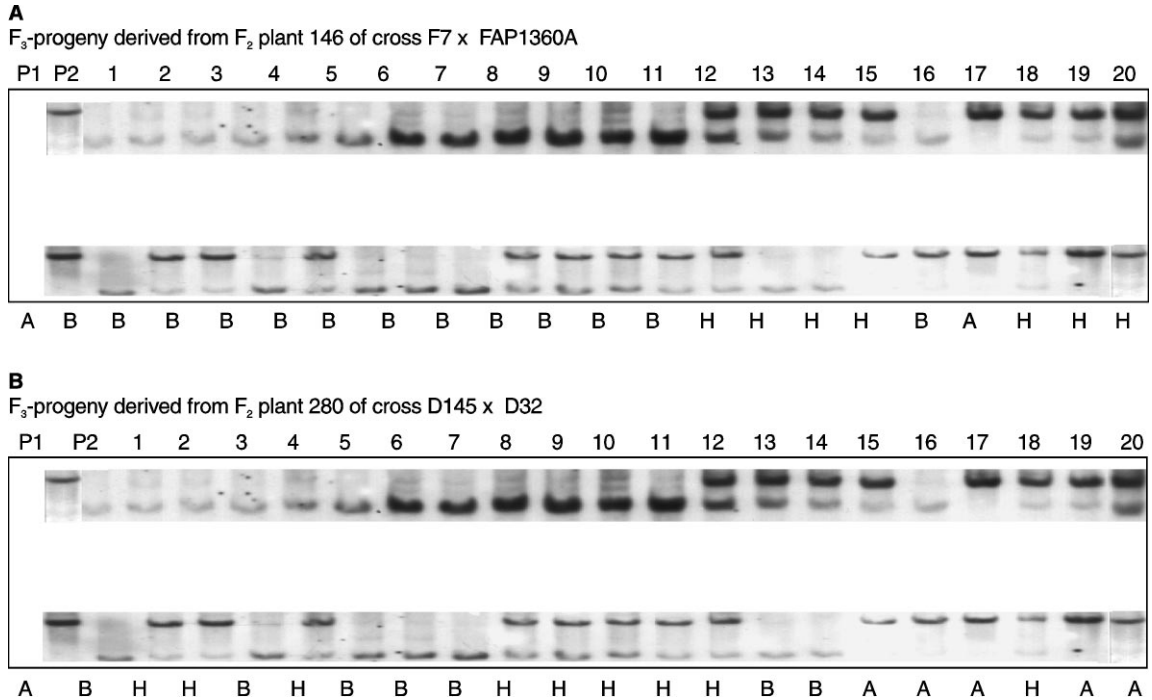

Fig. 2 RFLP banding patterns of marker *umc*10 in two F_3 lines from cross F7 × FAP1360A (**A**) and cross D145 × D32 (**B**). **A** Susceptible parental inbred F7 (*P1*), resistant parental inbred FAP1360A (*P2*), 13 resistant (*lanes 1–13*) and 7 susceptible (*lanes* 14–20) F_3 plants from F_2 plant no. 146 of cross F7 × FAP1360A. **B** Susceptible parental inbred D145 (*P1*), resistant parental inbred D32 (*P2*), 7 resistant (*lanes 1–7*) and 13 susceptible (*lanes 8–20*) F_3 plants from F_2 plant no. 280 of cross D145 × D32. *A* is homozygous for the RFLP band of the susceptible parent, *B* is homozygous for the RFLP band of the resistant parent, and *H* is heterozygous

timely inoculation of these tender plants results in higher disease incidence. Scott and Louie (1996) suggested that selection under rigorous conditions in the greenhouse results in lines having a high level of resistance to MDMV. Our results suggest that preliminary screening of a large number of genotypes for SCMV resistance could be done under controlled conditions in the greenhouse. Subsequently, the resistance of the selected materials must be confirmed in field conditions using artificial infection.

Detection of the *Scm*1 gene

All parental inbreds, D21, D32, FAP1360A, and Pa405 displayed complete resistance against SCMV in both greenhouse and field tests. Further tests indicated that in addition to SCMV, these inbreds also have complete resistance to MDMV, johnsongrass mosaic virus (JGMV), and sorghum mosaic virus (SrMV) (Kuntze et al. 1995). Our test of allelism indicated that the resistant European inbreds and Pa405 have at least one common genomic region, which confers resistance to SCMV. Three possible explanations concerning the common genomic region are: (1) the three resistant European inbreds all possess the *Mdm*1 gene, which confers resistance to MDMV in Pa405, (2) the inbreds D21, D32, and FAP1360 A carry a different allele at the *Mdm*1 locus having the same effect, and (3) all three European inbreds have a new gene tightly linked to the *Mdm*1 locus. Since (1) Pa405 has different RFLP banding patterns than the European inbreds for markers *umc*85 and *bnl*6.29 (data not shown) and (2) resistance genes are found in clusters in the maize genome (McMullen and Simcox 1995), we prefer to designate this gene as *Scm*1, even though we can not rule out that it is identical with *Mdm*1. Cloning of the gene(s) is necessary to resolve this issue.

Indications for additional genes

In many cases, resistance to plant viruses is under a simple genetic control involving a single dominant or recessive gene (Fraser 1990). However, there are numerous reports in the literature indicating that resistance to a particular virus is under a complex genetic control (McMullen et al. 1994; Caranta and Plaloix 1996). Segregation analyses in our study also suggested that more than one gene is required for complete resistance against SCMV.

Assuming that resistance to SCMV in three resistant European inbreds is controlled by a single gene, we first tried to fit the segregation for resistant and susceptible plants with a single-gene model. Deficiency of susceptible plants in the F_2 and backcross progenies in most of the crosses at Eckartsweier suggested the presence of

multiple genes for SCMV resistance. Segregation analyses supported a multigene model for resistance to SCMV. At Hohenheim, resistance in all crosses excluding F7 × FAP1360A fit well with a two-gene model (13r : 3s or 9r : 7s).

At Eckartsweier, segregation in three of the four F_2 and two of the three backcross generations supported a three-gene model: presence of any two genes in homozygous or heterozygous dominant conditions produced resistance to SCMV. Under greenhouse conditions, resistance in all crosses except KW1292 × FAP1360A seemed to be controlled by two genes. The presence of susceptible plants in a greater proportion in the F_1 generation and F_3 lines (#146) of cross F7 × FAP1360A and (#280) cross D145 × D32 in greenhouse experiments supports our hypothesis that under these conditions resistance to SCMV in FAP1360A and D32 is controlled by more than one gene.

Any comparison among various reports in the literature on the inheritance of SCMV or MDMV resistance is problematic because each study has used different testing conditions, virus strains and inoculation methods, susceptible parents, and scoring systems. The conclusions drawn in some of the studies pertaining to the genetic basis of resistance to MDMV were based on data from tests in the field or in greenhouse environments; some involved the use of a single strain of virus, whereas others used a mixture of strains (Louie et al. 1991). Mikel et al. (1984) studied resistance in Pa405 to a mixture of MDMV and SCMV and proposed a three-gene model, one gene being essential with either of the other two for complete resistance. In our study under greenhouse conditions, resistance in FAP1360A fits a two-gene model; however, the expression of the two genes was dependent on the susceptible parent (KW1292 and F7). Furthermore, the F_1 generation of some crosses developed more susceptible plants under greenhouse than under field conditions, indicating different degrees of dominance across different crosses and environments. McMullen and Louie (1989) also found that the resistance of Pa405 did not have the same degree of dominance in F_1 with K55 as with yM14. Similarly, the time of ratings and different scoring systems used to assess whether plants are resistant or susceptible may also result in different modes of inheritance. For instance, Mikel et al. (1984) observed a single-gene and two complementary gene control at earlier ratings and a three gene control at a final rating for resistance to MDMV in B68 and Pa405.

Molecular marker analyses for mapping of resistance genes

McMullen and Louie (1991) observed that the detection of multiple genes for virus resistance using segregation analysis alone is always suspect because of the difficulties in differentiating primary genetic effects from genotype × environment interactions. Therefore, we used molecular marker analyses to confirm and detect the gene(s) responsible for SCMV resistance in European maize inbreds. The *G*-test for markers *umc*85, *bnl*6.29, and *phi*075 confirmed the presence of a gene, here denoted as *Scm*1, on chromosome 6S for resistance to SCMV in the field experiments at Hohenheim for all four crosses (Table 3). With the exception of 1 individual for marker *umc*85, the absence of P1P1 genotypes for all other flanking markers in the class of resistant F_2 plants indicates that *Scm*1 is dominant. The incidence of 1 resistant individual in cross D408 × D21 with marker genotype P1P1 for marker *umc*85 but genotype P1P2 for the closely linked (map distance approx. 1 cM) marker *bnl*6.29 is most likely attributable to a rare cross-over event in one parental gamete between marker *umc*85 and *Scm*1. The absence of P1P2 and P2P2 genotypes at markers *umc*85 and *bnl*6.29 in the class of susceptible plants in crosses KW1292 × FAP1360A and D408 × D21 suggests that *Scm*1 is most likely the only active resistance gene segregating in these two crosses in the field test at Hohenheim. However, the hypothesis of a second recessive resistance gene segregating independently from *Scm*1 in these crosses, as suggested by the segregation ratios in the entire F_2 populations (Table 2), can not be ruled out. This is because under a two-gene model (segregation ratio 13r : 3s), one expects the occurrence of P1P1 genotypes, in the class of resistant genotypes, but with a sample size of $n = 10$, the probability of not observing one such individual is 0.45. Segregation in the F_3 lines derived from resistant F_2 plants homozygous or heterozygous for markers *umc*85 and *bnl*6.29 in cross KW1292 × FAP1360A could also not differentiate between these two hypotheses. Thus, a larger sample size would be required for marker assays to discriminate between them.

From the occurrence of P1P2 and P2P2 marker genotypes for markers *umc*85, *bnl*6.29, and *phi*075 in the class of susceptible plants in crosses F7 × FAP1360A and D145 × D32, one can infer the segregation of at least one additional resistance gene in these two crosses. This is consistent with the segregation ratios observed in the F_2 population of cross D145 × D32 in field as well as in greenhouse experiments and cross F7 × FAP 1360A in the greenhouse (Table 2) .

Evidence in support of additional resistance gene(s) in these two crosses was also obtained from RFLP analyses of susceptible and resistant plants of two F_3 lines (#146 of cross F7 × FAP1360A and #180 of cross D145 × D32) tested under greenhouse conditions. The *G*-test indicated the presence of a second resistance gene on chromosome 3 near marker *umc*10 (Table 3). Because all resistant F_3 plants in each F_3 line were either heterozygous (P1P2) or homozygous (P2P2) for the RFLP band of the resistant parent P2, we conclude that this resistance gene is dominant and designate it

*Scm*2. Both *Scm*1 and *Scm*2 are necessary for resistance in FAP1360A and D32, and segregation analyses in the field test at Hohenheim also indicated the complementary gene action of two dominant genes (9r : 7s segregation ratio) in cross D145 × D32. However, presence of all three marker genotypes for marker *umc*44 in the susceptible plants of F_3 line #280 indicate that the two genes are not sufficient for resistance to SCMV under greenhouse conditions. Segregation patterns of the F_3 lines derived from 5 susceptible and 8 resistant F_2 plants heterozygous for RFLP marker *umc*85 support the segregation of three resistance genes in cross F7 × FAP1360A under greenhouse conditions. In order to confirm these results and for fine mapping of the resistance genes identified in these crosses, further studies on the molecular mapping of SCMV resistance using large populations of F_3 lines are in progress in our laboratory.

By applying bulk segregant analysis, McMullen et al. (1994) identified three genetic loci (*Wsm*1, *Wsm*2, *Wsm*3) for resistance to wheat streak mosaic virus in maize inbred Pa405. *Wsm*1 is located on the short arm of chromosome 6 between RFLP loci *umc*85 and *bnl*6.29 near the nucleolus organizer region. This is the same region of chromosome 6 where gene *Mdm*1 is also located, but it is not known whether *Mdm*1 and *Wsm*1 are the same genes. *Wsm*2 is located on chromosome 3 near marker *umc*10 (1.7 cM), while *Wsm*3 was detected on chromosome 10 near marker *umc*44 (8.1 cM). In our study, the *G*-test for independent segregation of genotypes at RFLP marker *umc*44 did not support the presence of a third gene on chromosome 10 for resistance to SCMV. Ming et al. (1997) mapped a locus *Mv*1 for resistance to maize mosaic virus near the centromere of chromosome 3 between RFLP markers *umc*102 and *php*20508, with an estimated map distance of 4 cM and 6 cM, respectively. This map position is very close to the position of *Wsm*2 in Pa405 and *rp*3, a gene for resistance to *Puccinia sorghi*. They hypothesized that *Wsm*2, *Mv*1 and *rp*3 are not the same genes. Our findings on the position of *Scm*2 strengthen the conclusion of McMullen and Simcox (1995) that a cluster of genes for resistance to various maize pathogens including SCMV is located in this region on chromosome 3.

Conclusions

Our results suggest that either gene *Mdm*1 conferring resistance to MDMV in Pa405 or a new gene, *Scm*1, very tightly linked to *Mdm*1 is present in three European inbreds and Pa405 for SCMV resistance. This was evident from tests of allelism between resistant European lines and Pa405 and supported by molecular marker analyses. Besides, we mapped a second locus *Scm*2 near the centromere of chromosome 3. Segregation analyses and RFLP data on two F_3 families suggest that both *Scm*1 and *Scm*2 are not sufficient for complete resistance to SCMV in inbreds FAP1360A and D32. In addition to these two major genes, the occurrence of a small number of susceptible plants in F_1 and F_2 indicates the presence of one or more major or minor genes, the expression of which is highly influenced by the environmental conditions. Because of the latter and other factors, segregation analyses alone provide only weak indications about the number of genes contributing to SCMV resistance. Further marker-assisted investigations are necessary for resolving the complex genetic basis of SCMV resistance in the European maize germplasm.

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