Genetics of Cowpea Reactions to Two Strains of Cowpea Mosaic Virus from Tanzania

P. N. Patel

Legume breeder/pathologist, IITA/USAID/Tanzania Grain Legume Research Project, Crop Development Division, Ministry of Agriculture, P. O. Box 9071, Dar es Salaam, Tanzania.
Present address: Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India.
Contribution from the International Institute of Tropical Agriculture, Ibadan, Nigeria, and Crop Development Division, Ministry of Agriculture, Tanzania.
Grateful acknowledgement is made to P. C. Duffield and J. M. Liwenga for advice and encouragement and to Catherine Kuwite for technical assistance.
Accepted for publication 8 May 1981.

ABSTRACT

Patel, P. N. 1982. Genetics of cowpea reactions to two strains of cowpea mosaic virus from Tanzania. Phytopathology 72: 460-466.

Inheritance and genetic relationships among the four host reactions, resistant or immune (R), susceptible (S), necrotic local lesion resistant (NLLR), and lethal susceptible (LS), produced by two strains of the cowpea mosaic virus, CPMV-LL and CPMV-TN, were studied in F_1, F_2, F_3, and testcross populations from 82 crosses of cowpea (*Vigna unguiculata*). The crosses involved 15 R, seven S, seven NLLR, and four LS reacting cowpea lines. CPMV-LL induced R, NLLR, and S reactions. CPMV-TN induced R, LS, and S reactions. Plants in the segregating populations or of the parents classified as R or S, reacted similarly to both the strains, whereas those classed as NLLR to CPMV-LL were either S or LS in reaction to CPMV-TN. Reaction of F_1s and segregation patterns in the hybrid populations indicated that interaction of two dominant genes, designated *MVN* and *MVS*, governed the four reactions to the two strains. *MVN* controlled the necrotic (hypersensitive) reactions, NLLR and LS. *MVS* governed the S reaction and its recessive allele, *mvs*, controlled the R reaction. *MVN* was epistatic to *MVS*, but hypostatic to *mvs*. Three different necrosis genes (*MVN*-1, *MVN*-2, and *MVN*-3) were identified on the basis of appearance of the lesions and reactions to CPMV-TN. They involved the same locus. *MVN*-1 was dominant over *MVN*-2 and *MVN*-3, and *MVN*-3 was suspected of being dominant over *MVN*-2. All 15 R parents had the same recessive gene controlling the R reaction. The 29 parents crossed represented seven genotypes (one allele of each gene mentioned): *mvs mvn* (R), *mvs MVN*-1 (R), *mvs MVN*-3 (R), *MVN*-1 *MVS* (NLLR/LS), *MVN*-2 *MVS* (NLLR/S), *MVN*-3 *MVS* (NLLR/S), *mvn MVS* (S). From the similarity in the properties of the two strains reported earlier and the results of the present genetic studies, it is possible that CPMV-TN evolved in nature by a mutation from the CPMV-LL strain to overcome necrotic local lesion type of resistance controlled by the *MVN* gene in cowpeas.

Cowpea mosaic virus (CPMV) (14) in cowpea, *Vigna unguiculata* (L.) Walp., occurs widely in important cowpea-growing areas of Tanzania. Two pathogenic strains of the virus have been identified by differential reactions on cowpea cultivars (9). During 1977 and 1978, one of the strains was so destructive to plants of the high-yielding, widely adapted cultivar SVS-3 that it was withdrawn for further improvement in disease resistance. In a search for resistant parents for the ongoing cowpea breeding program, 258 accession lines, reported to possess multiple disease resistance in West Africa (Nigeria), were tested against the two Tanzanian CPMV strains. The reactions of these lines were classified in four main types: resistant or immune (R), necrotic local lesion resistant (NLLR), lethal susceptible (LS), and susceptible (S). Lines that were either R or S to one virus strain reacted

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0031-949X/82/05046007/$03.00/0

similarly to the other strain, whereas lines that produced necrotic local lesions to the CPMV-LL strain gave either an LS or S reaction to the CPMV-TN strain (8). Information on the genetic relationships among these reactions was important for increasing the efficiency of the ongoing multiple disease resistance cowpea breeding program and the incorporation of resistance to CPMV into high-yielding cultivars. The results obtained are presented here.

Inheritance of R and tolerant reactions to CPMV and other viruses in cowpea have been studied (3–6,10–13). The tolerant reaction to CPMV is governed by three additive homozygous genes and the moderately S, S, or highly S reactions result when two, one, and none, respectively, of these genes are present (3). The R reaction, expressed as necrotic local lesions in the inoculated primary leaves without any systemic symptoms (hypersensitive reaction) in cowpea, is controlled by single dominant genes in the case of CPMV (3), certain strains of cucumber mosaic virus (13), tobacco ring spot virus (5), and cowpea strains of southern bean mosaic virus (4). Allelic relationships among these genes have not been studied fully. Resistance to tobacco ring spot virus in cowpea is linked to cucumber mosaic virus susceptibility (6). One or two dominant genes govern tolerant or R reactions to chlorotic mottle virus in cowpea (3,12). A high level of R (immunity) to bean yellow mosaic virus in cowpea (10) is controlled by a single recessive gene.

MATERIALS AND METHODS

The cowpea lines (cultivars, breeding lines, and germ plasm lines are collectively referred to here as "lines"), their pedigrees, origins, and reactions to the CPMV-LL and CPMV-TN strains of the CPMV from Tanzania are listed in Table 1. The crosses involved 15 R-, seven S-, seven NLLR-, and four LS-reacting parents. Eighty-two crosses, which represented the four cowpea reactions to the virus strains in all possible combinations, were studied. The F_1 and F_2 generations were grown in the field or screened pothouse. Observed segregation patterns in the F_2 populations were confirmed by using representative F_3 families and testcrosses.

For studying the reactions to CPMV, the hybrid populations were grown in a screened pothouse in 2-L plastic pots filled with unsterilized topsoil from virgin land. Depending on the generation, one to eight plants were grown per pot. Parent plants were similarly raised as checks. The test plants were inoculated with CPMV-LL and CPMV-TN by rubbing the primary leaves, before they were fully expanded, with a forefinger wetted with the inoculum. Carborundum powder (22-μm, 600-mesh), used as an abrasive, was either placed in the inoculum or dusted on the leaves to be inoculated. Plants that developed no symptoms were reinoculated. Inoculum was prepared by grinding the infected cowpea leaves and diluting the expressed sap 1:5 (v/v) with 0.01 M neutral phosphate buffer. When inoculation with a mixture of CPMV-LL and CPMV-TN strains was desired, equal numbers of leaves infected with each strain were ground together and the sap was diluted 1:5 as before.

Pure cultures of the strains were obtained by three successive single-lesion transfers on *Chenopodium quinoa*, or by passage through differentially reacting cowpea cultivars. Purity of the isolates was checked periodically on plants of lines SVS-3 and TVu 410. Strain CPMV-TN was maintained and propagated in plants of line TVu 410 and strain CPMV-LL in plants of cultivar Prima or line TVu 43.

Back-inoculations from the symptom-free plants were done in some of the critical crosses and parents only. The sap expressed from the third or fourth trifoliolate leaves was inoculated on TVu 410, Prima, or both, as described above.

Observations on the reactions in the hybrid populations and parents were recorded two to three times between 5 and 28 days after inoculation.

Reactions in the hybrid cowpea populations. R, S, NLLR, or LS reactions were obtained in the parents, F_1s, and segregating cowpea populations. The characteristics of the reactions were: R = no symptom in the inoculated primary or trifoliolate leaves, and the virus was not detected in the trifoliolate leaves in back-inoculations; S = chlorotic spots and areas in the primary leaves and systemic mottling of the trifoliolate leaves; NLLR = necrotic local lesions (ranging from pinpoint to 1.0 mm in diameter) in the inoculated primary leaves, no systemic symptoms or detectable virus in the trifoliolate leaves; LS = chlorotic or large necrotic spots (2–4 mm in diameter) in the inoculated primary leaves, and systemic necrosis in the growing point, leading to complete collapse of the seedlings. Of these, the NLLR and LS reactions are considered to be hypersensitive responses because rapid necrosis develops in the inoculated seedlings. The letters R and S (meaning resistant and susceptible) were added to the basic necrotic local lesion and lethal reactions to indicate their effects on the plant growth and productivity. The genes governing these reactions are often referred to in the text that follows as "necrosis genes."

The F_1 and the F_2 plants produced R, S, or NLLR reactions when inoculated with CPMV-LL and R, S, or LS reactions in inoculations with CPMV-TN. Depending on the parents involved in a given cross, one, two, or all three reaction classes were obtained in F_2 populations. Phenotypically, the reactions in the parents and in the hybrid populations were similar.

A few NLLR-reacting plants developed deformed trifoliolate leaves with very faint mottling and a systemic necrosis that sometimes killed the growing point. The plants remained stunted. The virus could be recovered from the deformed trifoliolate leaves. These symptoms often developed when the primary leaves were too young at the time of inoculation and infection with the virus became systemic. For determining the segregation ratios, they were classified as NLLR.

RESULTS

Reactions in the F_1 plants and the suggested segregation ratios for the reactions of plants in the F_2, F_3, and testcross progenies inoculated with CPMV-LL and CPMV-TN strains, separately or in mixture, are presented in Tables 2 to 5. Of the 82 crosses studied,

TABLE 1. Pedigree, origin, and reactions to two cowpea mosaic virus (CPMV) strains of the cowpea lines used to investigate the genetics of resistance and susceptibility

Cowpea lines	Pedigree	Origin	Reaction[a] to CPMV strain: LL	TN
TVu 227	Attracto	Nigeria	R	R
TVu 317	C. 5810-6	USA	R	R
TVu 345	Tanong-1	Nigeria	R	R
TVu 612	Farin Dengi-2	Nigeria	R	R
TVu 1190	V.U. 5	Kenya	R	R
TVu 1948	PI 186454	Nigeria	R	R
TVu 2331	Chaula	India	R	R
TVu 2480	Unknown		R	R
TVu 4536	IF H 79-2	Nigeria	R	R
TVu 4539	IF H 53-1	Nigeria	R	R
TVu 4540	IF H 27-8	Nigeria	R	R
TVu 4544	IF H 113-1	Nigeria	R	R
TVu 6666	IF H 38-2	Nigeria	R	R
TVx 1836-481	TVu 1190 × Prima	Nigeria	R	R
TVx 1836-348	TVu 1190 × Prima	Nigeria	R	R
TVu 266-1	Paraguay 18	USA	NLLR	S
TVu 408	IC 2238 C	USA	NLLR	S
TVu 410	Texas Purple Hull	USA	NLLR	S
SVS-3	Selection	Tanzania	NLLR	LS
TVu 201	Outcross selection	Nigeria	NLLR	LS
TVu 222	Paraguay 1 A	USA	NLLR	LS
TVu 1564-P_2	Chinese Red × Iron	USA	NLLR	LS
TVu 2616	FC 31660	USA	S	S
TVu 76	Prima	Nigeria	S	S
5/8/2/2	Breeding line	India	S	S
1/2/3	Breeding line	India	S	S
P_{33}-2B	Breeding line	India	S	S
P_{33}-1C	Breeding line	India	S	S
CP/2	Selection	Tanzania	S	S

[a] R = resistant, NLLR = necrotic local lesion resistant, S = susceptible, and LS = lethal susceptible.

the detailed results from only a few crosses which represent the different segregation patterns observed, are given in these tables; others that segregated similarly are mentioned in the text. Since the inheritance patterns of the crosses and their reciprocals were the same, data from only one are presented. It also indicated that these reactions are governed by nuclear genes.

S/S × NLLR/LS (one parent reacting NLLR to CPMV-LL and LS to CPMV-TN and the other parent reacting S to both the CPMV strains). In the cross SVS-3 × 5/8/2/2 (Table 2), inoculation with CPMV-LL induced the NLLR reaction in F_1 plants and F_2 plants segregated 3 NLLR:1 S, suggesting that NLLR reaction of SVS-3 was controlled by a single dominant gene. The reaction to CPMV-TN was LS in F_1 plants. In the F_2, a segregation ratio of 3 LS:1 S was observed. Thus, the LS reaction was also dominant over the S reaction and was governed by a single gene (Table 2). Prima and TVu 2616 also reacted like 5/8/2/2.

The single-dominant-gene hypothesis for NLLR and LS reactions was also confirmed by the segregation ratio of 3 NLLR:1 S

TABLE 2. Reactions of F_1s and segregation for reactions to CPMV-LL and CPMV-TN strains in F_2 populations of cowpea crosses

Crosses	Strain	Reaction F_1	No. of F_2 plants reacting:[a] NLLR	LS	S	R	Suggested ratio	χ^2 value	*P*
S/S × NLLR/S									
5/8/2/2 × TVu 408	LL	NLLR	75	0	23	0	3 NLLR:1 S	0.12	0.75–0.50
	TN	S	0	0	89	0	0 NLLR:1 S	0.0	1.0
S/S × NLLR/LS									
SVS-3 × 5/8/2/2	LL	NLLR	122	0	50	7	3 NLLR:1 S	0.83	0.50–0.25
	TN	LS	0	113	37	0	3 LS:1 S	0.03	0.90–0.75
S/S × S/S									
5/8/2/2 × Prima	LL	S	0	0	84	0	0:1 S	0.0	1.0
	TN	S	0	0	104	0	0:1 S	0.0	1.0
NLLR/S × NLLR/LS									
TVu 408 × SVS-3	LL	NLLR	234	0	0	0	1 NLLR:0 S	0.0	1.0
	TN	LS	0	144	41	0	3 LS:1 S	0.87	0.50–0.25
NLLR/S × NLLR/S									
TVu 410 × TVu 266-1	LL	NLLR	298	0	0	11	1 NLLR:0 S	0.0	1.0
	TN	S	0	0	108	0	0 NLLR:1 S	0.0	1.0
NLLR/LS × NLLR/LS									
TVu 222 × SVS-3	LL	NLLR	175	0	0	0	1 NLLR:0 S	0.0	1.0
	TN	LS	0	133	0	0	1 LS:0 S	0.0	1.0
TVu 1564-P_2 × SVS-3	TN	LS	0	179	38	0	3 LS:1 S	6.49	0.01–.001
R/R × S/S									
TVu 612 × 5/8/2/2	LL	S	0	0	90	31	3 S:1 R	0.02	0.90–.75
	TN	S	0	11	219	68	3 S:1 R	0.82	0.50–.25
TVu 317 × 5/8/2/2	LL	NLLR	119	0	38	47	9 NLLR:3 S:4 R	0.47	0.99–.95
	TN	S	0	0	84	22	3 S:1 R	1.14	0.50–.25
TVu 345 × 5/8/2/2	LL	NLLR	179	0	73	72	9 NLLR:3 S:4 R	3.69	0.25–.10
	TN	S	0	45	266	109	3 S:1 R	0.20	0.75–.50
TVu 1190 × 5/8/2/2	LL	NLLR	141	0	47	69	9 NLLR:3 S:4 R	0.47	0.90–.75
	TN	LS	0	105	39	32	9 LS:3 S:4 R	4.66	0.10–.05
R/R × NLLR/LS									
TVu 227 × SVS-3	LL	NLLR	37	0	15	23	9 NLLR:3 S:4 R	1.66	0.50–.25
	TN	LS	0	104	40	29	9LS:3S:4R	6.92	0.05–.02
TVu 317 × SVS-3	LL	NLLR	141	0	2	43	3 NLLR:1 R	0.35	0.75–.50
	TN	LS	0	146	38	50	9LS:3S:4R	3.59	0.25–.10
TVu 345 × SVS-3	LL	NLLR	59	0	2	32	3 NLLR:1 R	4.39	0.02–.01
	TN	LS	0	313	48	114	9 LS:3S:4R	10.60	0.005
TVu 1190 × SVS-3	LL	NLLR	118	0	0	37	3 NLLR:1 R	0.14	0.75–.50
	TN	LS	0	694	0	229	3 LS:1 R	0.02	0.90–.75
R/R × NLLR/S									
TVu 410 × TVu 317	LL	NLLR	246	0	11	106	3 NLLR:1 R	3.41	0.10–.05
	TN	S	0	13	273	93	3 S:1 R	0.04	0.90–.75
TVu 1190 × TVu 408	LL	NLLR	131	0	0	53	3 NLLR:1 R	1.33	0.25–.10
	TN	LS	0	120	31	58	9 LS:3S:4 R	2.58	0.50–.25
TVu 408 × TVu 227	LL	NLLR	33	0	12	22	9 NLLR:3 S:4 R	2.21	0.50–.25
	TN	S	0	0	20	8	3 S:1 R	0.19	0.75–.50

[a]NLLR = necrotic local lesions resistant, LS = lethal susceptible, S = susceptible, and R = resistant.

TABLE 3. Segregation for reactions to CPMV-LL and CPMV-TN in heterozygous F_3 families of three cowpea crosses

Crosses	Strain	No. of heterozygous families screened	No. of plants reacting:[a] NLLR	LS	S	R	Expected ratio	χ^2 value	*P*
TVu 410 × CP/2	LL	11	348	0	127	0	3 NLLR:1 S	0.72	0.50–0.25
	TN	11	0	0	350	0	0 NLLR:1 S	0.00	1.0
SVS-3 × 5/8/2/2	LL	15	263	0	81	0	3 NLLR:1 S	0.42	0.75–0.50
	TN	6	0	197	67	0	3 LS:1 S	0.01	0.95
TVu 612 × 5/8/2/2	LL	4	0	0	128	43	3 S:1 R	0.28	0.75–0.50
	TN	6	0	0	304	78	3 S:1 R	4.23	0.05–0.02

[a]NLLR = necrotic local lesion resistant, LS = lethal susceptible, S = susceptible, and R = resistant.

in response to virus strain CPMV-LL and 3 LS:1 S ratio in response to virus strain CPMV-TN in F_3 plants of the heterozygous F_3 families of SVS-3 × 5/8/2/2 (Table 3). The F_3 families common in the two inoculations were heterozygous for reaction to both virus strains.

It is possible that the NLLR and LS reactions in SVS-3 inoculated with CPMV-LL and CPMV-TN, respectively, are governed by the same dominant gene. This necrosis gene is referred to as the *MVN*-1 gene.

S/S × NLLR/S (one parent reacting S to both of the CPMV strains and the other parent reacting NLLR to CPMV-LL and S to CPMV-TN). In cross 5/8/2/2 × TVu 408, inoculations with CPMV-LL produced the NLLR reaction in F_1 and F_2 plants segregated in 3 NLLR:1 S ratio indicating that the NLLR reaction in TVu 408 was dominant over the S reaction in 5/8/2/2 (Table 2). This single-dominant-gene hypothesis was also confirmed by a 3 NLLR:1 S ratio among the plants of the heterozygous F_3 families of a similar cross, TVu 410 × CP/2 (Table 3).

In response to CPMV-TN, the F_1 plants and the plants in the F_2 population gave the S reaction. All the plants of the heterozygous F_3 families of the cross TVu 410 × CP/2 (Table 3) also produced the S reaction when inoculated with CPMV-TN. The results showed that the gene governing the NLLR reaction does not react to CPMV-TN, and the S plants in F_2 populations inoculated with CPMV-TN represented only one phenotypic, but three genotypic, classes. This necrosis gene is referred to as the *MVN*-2 gene. TVu 266-1 and TVu 410 behaved like TVu 408 and Prima, CP/2, and TVu 2616 like 5/8/2/2 in the four additional crosses that were studied.

S/S × S/S. All the F_1 and F_2 plants of cross 5/8/2/2 × Prima (Table 2) reacted as S, indicating that the gene governing the S reaction in the parents to both strains was the same.

NLLR/S × NLLR/LS. In the cross TVu 408 × SVS-3, the F_1 plants and all the F_2 plants produced NLLR reaction to CPMV-LL suggesting that the dominant necrosis genes (*MVN*-1 and *MVN*-2) governing the NLLR reaction in these lines are at the same locus (Table 2).

The F_1 plants inoculated with CPMV-TN produced LS reaction and the F_2 plants segregated in 3 LS:1 S ratio. When the indications from the segregation patterns in the previous crosses are considered, and as the *MVN*-1 and *MVN*-2 genes are at the same locus as observed in inoculations with virus strain CPMV-LL in the present cross, the 3 LS:1 S ratio in response to virus strain CPMV-TN would indicate that the *MVN*-1 gene in SVS-3 is dominant over the *MVN*-2 gene in TVu 408. TVu 266-1 and -410 behaved like TVu 408.

NLLR/S × NLLR/S. The plants in the F_1 and F_2 generations in the cross TVu 410 × TVu 266-1 (Table 2) produced NLLR reaction to virus strain CPMV-LL and S reaction to virus strain CPMV-TN, showing that the *MVN*-2 gene in both the parents was the same.

In some of the crosses inoculated with CPMV-LL, when all the plants were expected to give the NLLR reaction or segregate into NLLR:S classes, a few plants did not develop any reaction. It was observed that the development of necrotic local lesions on the primary leaves was affected by factors such as age and physiological condition of the leaves at the time of inoculation, concentration of the virus in the sap, and the pressure applied in inoculations. Under the experimental conditions, best results were obtained in inoculations done after the primary leaves had completely unfolded, but before they had fully expanded. Necrotic local lesions were readily produced in TVu 266-1, -408, and -410 lines compared to that in SVS-3.

NLLR/LS × NLLR/LS. In the cross TVu 222 × SVS-3 (Table 2), the F_1 and all the F_2 plants produced NLLR reaction to CPMV-LL and LS reaction to CPMV-TN, suggesting that the *MVN*-1 gene in both the parents was the same.

A second cross studied in this group was TVu 1564-P_2 × SVS-3 (Table 2). TVu 1564-P_2 produces minute (up to 0.5-mm diameter) necrotic local lesions when inoculated with CPMV-LL and large necrotic and/or ring spot (2–4 mm in diameter) and death of the seedlings when inoculated with CPMV-TN. In the cross with SVS-3, inoculations with CPMV-TN produced LS reaction in F_1, but the large chi-square value for the F_2 suggested a possible deviation from the expected 3:1 ratio. There were more plants in the LS category. Most of the LS plants developed ring spots and necrotic spots on the primary leaves before death. Of the remaining plants classed as S, some showed similar ring spots and necrotic spots, but all were stunted and carried heavily puckered and deformed trifoliolate leaves. The differential reaction suggested that the gene

TABLE 4. Segregation for reaction to CPMV-TN in six cowpea test-crosses

Crosses	No. of plants tested	No. of plants reacting:[a] R[a]	S	LS	Expected ratio R:S:LS	χ^2 value	*P*
TVu 612 × F_1 (P_{33}-1C × TVu 612)	30	15	15	0	1:1:0	0.00	1.00
TVu 345 × F_1 (TVu 345 × 5/8/2/2)	70	34	36	0	1:1:0	0.02	0.90
TVu 1190 × F_1 (TVu 1190 × 5/8/2/2)	48	23	3	22	1:0:1	0.02	0.90
TVu 227 × F_1 (TVu 227 × SVS-3)	65	32	13	20	2:1:1	1.54	0.50–0.25
TVu 1948 × F_1 (TVu 201 × TVu 1948)	15	7	3	5	2:1:1	0.60	0.75–0.50
TVu 1190 × F_1 (TVu 1190 × SVS-3)	103	44	0	59	1:0:1	1.02	0.50–0.30

[a] R = resistant, S = susceptible, and LS = lethal susceptible.

TABLE 5. Segregation in eight F_2 cowpea populations for reaction to inoculations with a mixture of CPMV-LL and CPMV-TN

Crosses	No. of plants inoculated	No. of plants reacting:[a] NLLR[a] LS	NLLR S	S S	R R	Expected ratio	χ^2 value	*P*
TVu 317 × 5/8/2/2	175	4	100	36	35	9 NLLR-S:3 S:4 R	2.34	0.50–0.25
TVu 612 × 5/8/2/2	121	0	0	92	29	3 S:1 R	0.11	0.75–0.50
TVu 1190 × 5/8/2/2	184	116	0	29	39	9 NLLR-LS:3 S:4 R	3.46	0.25–0.10
TVu 317 × SVS-3	186	120	21	2	43	9 NLLR-LS:3 NLLR-S:4 R	4.47	0.25–0.10
TVu 612 × SVS-3	184	111	0	33	40	9 NLLR-LS:3 S:4 R	1.39	0.50–0.25
TVu 1190 × SVS-3	468	353	0	0	115	3 NLLR-LS:1 R	0.06	0.90–0.75
TVu 1190 × TVu 410	388	204	65	0	119	9 NLLR-LS:3 NLLR-S:4 R	6.75	0.05–0.02
TVu 408 × TVu 4540	105	0	60	23	22	9 NLLR-S:3 S:4 R	1.26	0.75–0.50

[a] Combinations of reactions: NLLR-LS = necrotic local lesions on the inoculated primary leaves and developed lethal susceptible reaction later; NLLR-S = necrotic local lesions of the inoculated leaves and systemic mosaic in the trifoliolates; S = susceptible; and R = resistant.

governing NLLR reaction in TVu 1564-P_2 is different from the *MVN*-1 and *MVN*-2 genes referred to earlier.

R/R × S/S. In the cross TVu 612 × 5/8/2/2 (Table 2) separate inoculations with CPMV-LL and CPMV-TN produced the S reaction in F_1 plants and the segregation ratio of 3 S:1 R was found in F_2 populations. Thus, the S reaction was dominant over the R reaction, and both the reactions were controlled by the same gene. This was further confirmed by a ratio of 3 S:1 R to both CPMV-LL and CPMV-TN in the heterozygous F_3 families of TVu 612 × 5/8/2/2 (Table 3), and by a ratio of 1 R:1 S in the testcross TVu 612 × F_1 (P_{33}-1C × TVu 612) inoculated with CPMV-TN (Table 4). Similar testcrosses with TVu 4540 as R/R parent and 5/8/2/2 or 1/2/3 as S/S parent also gave 1 R:1 S ratios. This gene is referred as MVS gene TVu 227, -4540, and -4544 lines behaved like TVu 612 and Prima and TVu 2616 like 5/8/2/2.

In TVu 317, -345, and -1190 × 5/8/2/2 crosses (Table 2), CPMV-LL produced NLLR reaction in F_1s and a ratio of 9 NLLR:3 S:4 R was obtained in the F_2 populations indicating that each of these R/R parents possessed a dominant necrosis gene besides the *MVS* gene, and the dihybrid ratio involved an interaction of the necrosis gene and the *MVS* gene with recessive epistasis. The necrotic local lesions produced in the F_1s and some plants in the F_2 populations showed phenotypic differences (described in the discussion).

When inoculated with CPMV-TN, the TVu 317 and -345 × 5/8/2/2 crosses produced S reaction in F_1 plants and gave a segregation ratio 3 S:1 R in the F_2 populations suggesting that only the *MVS* gene was operative (Table 2). This was also confirmed by 1 R:1 S ratio in the testcross, TVu 345 × F_1 (TVu 345 × 5/8/2/2) (Table 4). Many of the S plants developed large necrotic spots and/or ring spots (2–4 mm in diameter) together with chlorotic spots in the inoculated primary leaves. A few of these plants died later. Thus, the necrosis gene present in the TVu 317 and -345 lines produced effects distinct from those produced by the *MVN*-1 and *MVN*-2 genes described earlier. It is referred to as *MVN*-3 gene. The same gene may be present in TVu 1564-P_2.

In the cross TVu 1190 × 5/8/2/2, CPMV-TN produced LS reaction in F_1 hybrid and F_2 plants segregated in 9 LS:3 S:4 R ratio showing that the dominant necrosis gene interacting with the *MVS* gene in TVu 1190 was similar to the *MVN*-1 gene in SVS-3. Interaction of these two genes was confirmed by the segregation ratio of 1 R:1 LS in the testcross TVu 1190 × F_1 (TVu 1190 × 5/8/2/2) (a testcross for the *MVS* gene but a backcross for the *MVN*-1 gene) (Table 4). In similar testcrosses, TVx 1836-481 and TVx 1836-348 behaved like TVu 1190 and TVu 2616 like 5/8/2/2.

R/R × NLLR/LS. In the cross TVu 227 × SVS-3 (Table 2), the F_1 plants produced NLLR reaction to CPMV-LL and LS reaction to CPMV-TN. In the F_2 population segregation ratios of 9 NLLR:3 S:4 R to CPMV-LL and 9 LS:3 S:4 R to CPMV-TN confirmed the expected interactions of the *MVS* and *MVN*-1 genes. TVu 612, -1948, -2331,-2480, -4539, and -6666 behaved like TVu 227. The testcrosses, TVu 227 × F_1 (TVu 227 × SVS-3) and TVu 1948 × F_1 (TVu 201 × TVu 1948) (testcross for the *MVS* and *MVN*-1 genes) inoculated with CPMV-TN gave a ratio close to 2 R:1 S:1 LS (Table 4). The appearance of S and LS reacting plants in equal proportions confirmed that SVS-3 carries the *MVS* and *MVN*-1 genes in homozygous dominant condition.

In the F_2 of the TVu 317 and -345 × SVS-3 crosses, segregation ratios of 3 NLLR:1 R to CPMV-LL and 9 LS:3 S:4 R to CPMV-TN were obtained (Table 2). This indicated that the *MVN*-3 gene in the TVu 317 and -345 parents and the *MVN*-1 gene in SVS-3 were at the same locus, but the segregation to CPMV-TN suggested that the *MVN*-1 gene was dominant over the *MVN*-3 gene.

The F_2 of TVu 1190 × SVS-3 gave 3 NLLR:1 R ratio to CPMV-LL and 3 LS:1 R ratio to CPMV-TN, confirming that the dominant *MVN*-1 gene in TVu 1190 was identical to the one present in SVS-3. This was further substantiated by the segregation ratio of 1 R:LS in the testcross TVu 1190 × F_1 (TVu 1190 × SVS-3) (Table 4). Similar testcross with TVx 1836-481 as the parent gave the same ratio.

R/R × NLLR/S. Detailed results from three of the 13 crosses are presented in Table 2. The segregation patterns confirmed the expected interactions of the *MVS* gene with the necrosis genes, and that the *MVN*-2 gene in the TVu 266-1, -408, and -410 parents did not react to CPMV-TN.

In F_2 population of TVu 410 × TVu 612 (Table 2), the segregation ratios of 9 NLLR:3 S:4 R to CPMV-LL and 3 S:1 R to CPMV-TN were obtained. In the other crosses TVu 1948, -4536, -4539, -4540, -4544, and -6666 behaved like TVu 612 and TVu 266-1 and -410 like TVu 408.

In similar crosses involving TVu 317 and -1190 as R parents, the segregation ratios of 3 NLLR:1 R to CPMV-LL in both crosses, 3 S:1 R to CPMV-TN in TVu 317, and 9 LS:3 S:4 R to CPMV-TN in TVu 1190 crosses indicated that the *MVN*-3 gene present in TVu 317, the *MVN*-1 gene in TVu 1190, and the *MVN*-2 gene in TVu 410 are at the same locus, but behave distinctly with respect to phenotypic characters of the lesions and reaction to CPMV-TN. Results of previous crosses have already suggested that the *MVN*-1 gene in SVS-3 (which is identical to the one in TVu 1190) is dominant over the *MVN*-2 gene in TVu 410 and *MVN*-3 gene in TVu 317. The LS reaction of the F_1 and the segregation ratio in F_2 of TVu 410 × TVu 1190 (Table 2) also confirmed that the *MVN*-1 gene in TVu 1190 was dominant over *MVN*-2 gene in TVu 410. It was observed, however, that the LS reaction in the F_1 plants and in some F_2 plants was much delayed. Instead of the total collapse of the seedlings within 8–10 days after inoculation, as in the SVS-3 parent, such seedlings continued to survive longer with necrosis in the stem, small heavily puckered trifoliolate leaves that became brittle and easily defoliated, and with severely stunted growth. This effect may be due to interactions between the dominant alleles of the two necrosis genes, *MVN*-1 from TVu 1190 and *MVN*-2 from TVu 410, when present together as in F_1 and similar plants in F_2. Some effects of the presence of *MVS* gene cannot be ruled out. Similar responses also were observed in TVu 1190 × TVu 408 or -266-1 crosses.

Distinctive phenotypic characters of the lesions produced by *MVN*-2 gene in TVu 410 and the *MVN*-3 gene in TVu 317 are described in the discussion section of this article. In the F_1 plants of the cross TVu 410 × TVu 317 inoculated with CPMV-LL, the lesions were intermediate in size and color, and the segregation pattern in the F_2 indicated that the *MVN*-3 gene in TVu 317 may be dominant or partially dominant over the *MVN*-2 gene in TVu 410.

R/R × R/R. Allelic relationships among the genes governing R reactions were examined in crosses TVu 612 and eight other R parents (TVu 227, -317, -345, -1948, -2480, -4536, and -4539) and between TVu 1190 and nine other R parents (TVu 227, -317, -345, -2480, -612, -2331, -4536, -4540, and -6666). A cross between TVu 345 and TVu 317 also was studied.

All the plants in the F_2 populations of the crosses were R, suggesting that the same *MVS* gene was present in these R parents.

Inoculations of F_2 populations with a mixture of CPMV-LL and CPMV-TN. In the 70 crosses studied above, the relationships among the R, S, NLLR, and LS reactions in the host and two strains of the virus were inferred from the reaction in F_1 and F_2 populations inoculated either with CPMV-LL or CPMV-TN. To obtain direct evidence for the relationships, F_2 populations of 25 crosses were inoculated with a mixture of CPMV-LL and CPMV-TN. Earlier it was determined that these strains do not interact with each other in cowpea plants when inoculated together. The detailed results from eight crosses are presented in Table 5.

As expected, only four combinations of reactions NLLR/LS, NLLR/S, R/R, and S/S to the mixed inoculum were obtained. In the NLLR/LS combination, the plants developed NLLR reaction in the primary leaves 3–4 days after inoculation and typical LS reaction 8–10 days later; in NLLR/S combination the plants exhibited NLLR reaction followed by systemic mottling in the trifoliolate leaves. S/S or R/R reactions were the same as described before. The NLLR/LS combination was found only in crosses involving SVS-3 and TVu 1190; NLLR/S in crosses with TVu 266-1, -317, -345, -408, and -410, confirming earlier interpretation that NLLR/LS and NLLR/S were strain-specific host manifestations governed by the same gene in the host. The separation of the NLLR/LS, NLLR/S from S/S reacting plants made it possible to further confirm that the *MVN*-1 genes in lines TVu 1190 and SVS-3 were identical and dominant over the *MVN*-3

gene in TVu 317, -345, and *MVN*-2 gene in TVu 410, -266-1, and -408.

S- and/or R-reacting plants appeared in all the crosses. The segregation ratios in all the crosses followed the combinations of patterns obtained when the virus strains were inoculated individually and confirmed that two dominant genes with recessive epistasis were involved.

Segregation for hypersensitive:nonhypersensitive reacting plants. NLLR and LS are hypersensitive reactions and S and R nonhypersensitive reactions. When the proportion of NLLR or LS to R and S plants in crosses giving 9:3:4 ratios was calculated, it did not deviate from a ratio of 9 hypersensitive/7 nonhypersensitive expected ratio. This also confirmed that two dominant genes were involved and the homozygous recessive of either gene was epistatic to the effects of the other.

DISCUSSION

The results presented above confirmed my earlier observation of genetic relationships between the reactions in the cowpea cultivars to the CPMV-LL and CPMV-TN strains of the cowpea mosaic virus (8). It is proposed that two dominant genes, designated as *MVN* and *MVS*, in the host are responsible for the four reactions. *MVN* governs the strain-specific necrotic (hypersensitive) reactions (NLLR and LS) and *MVS* governs the S reaction when dominant and the R reaction when the recessive allele is homozygous. *MVN* is epistatic to *MVS* but *mvs mvs* is epistatic to *MVN*. The NLLR or LS reactions occur when the *MVN* and *MVS* genes are either homozygous dominant or heterozygous; S reaction is expressed when the *MVN* gene is homozygous recessive (*mvn mvn*) and the *MVS* gene is homozygous dominant or heterozygous; and the R reaction develops when the plants have *mvs mvs* alleles irrespective of the condition of *MVN*. Ali (1) reported a similar inheritance pattern in a cross of two resistant bean varieties, one with a dominant gene controlling hypersensitivity and the other with a recessive gene that controlled the immunity against a bean common mosaic virus strain.

Bliss and Robertson (3) reported that a single dominant gene controlled a NLLR reaction to CPMV in a selection from the cowpea cultivar Dixielee. In the present studies, a similar inheritance pattern was observed in five NLLR-reacting cowpea lines. Three resistant lines, TVu 317, -345, and -1190 also carried *MVN* gene besides *mvs mvs*. Three distinct necrosis genes (which were designated *MVN*-1, *MVN*-2, and *MVN*-3) were identified. They involved the same locus, but produced lesions differing in size and color in plants inoculated with CPMV-LL, and gave distinct reactions to CPMV-TN as described below:

MVN-1—Necrotic lesions large (0.5–1.0 mm in diameter) and red; LS reaction to CPMV-TN; dominant over *MVN*-2 and *MVN*-3; present in cowpea lines SVS-3, TVu 201, -222, and -1190.

MVN-2—Necrotic lesions like those produced by *MVN*-1; does not interact with CPMV-TN; recessive to *MVN*-1 and *MVN*-3; present in TVu 266, -408, and -410.

MVN-3—Necrotic lesions minute (up to 0.5 mm in diameter) and mixture of red and white; to CPMV-TN some plants develop large necrotic, chlorotic, and/or ring spots (2–4 mm in diameter) in the primary leaves leading to death in some plants, but mostly systemic mottling, severe puckering and deformation in the trifoliolate leaves, and stunting in plants; suspected of being completely or partially dominant over *MVN*-2; present in TVu 317 and -345 and possibly in TVu 1564-P_2.

Results from the inoculations of F_2 populations with a mixture of CPMV-LL and CPMV-TN strains demonstrated conclusively that NLLR/LS and NLLR/S reactions are the result of strain-specific manifestations of the necrosis genes *MVN*-1, *MVN*-2, or *MVN*-3, whereas R and S reactions are the result of nonstrain-specific manifestations of the recessive and dominant alleles of the gene *MVS*; the necrosis genes are the only basis for differential reactions of the cowpea lines to CPMV-LL and CPMV-TN. It is, therefore, suggested that these CPMV strains also have the corresponding pathogenicity genes. The gene in CPMV-LL interacts with *MVN*-1, *MVN*-2, and *MVN*-3 genes in cowpeas and produce NLLR reactions whereas the gene in CPMV-TN produces LS reaction in interaction with *MVN*-1 gene, does not interact with *MVN*-2 gene, or produces distinct effects when interacts with *MVN*-3 gene. As these necrosis genes involve the same locus in cowpeas, it is possible that one of these CPMV strains may be a natural mutant of the other strain. Evolution of CPMV-TN from CPMV-LL would appear to be more favorable for the virus since overcoming the NLLR-type of host resistance controlled by *MVN* genes against CPMV-LL strain would permit unlimited multiplication and systemic movement of such a mutant in cowpeas (carrying genes *MVN*-2 or *MVN*-3) and help the survival and spread via infected seed. This was also indicated from the survey of sap-transmissible viruses in the area of Tanzania where both the virus strains are widely prevalent (9). All the naturally affected plants of cowpeas (mixture of land-races) in the farmers field, the samples from which yielded CPMV-LL and/or CPMV-TN strains, had systemic mosaic symptoms without any necrosis. Natural selection had probably eliminated cowpeas carrying the *MVN*-1 gene. Therefore, when cultivar SVS-3 having this gene was introduced in the area, the prevalence of CPMV-TN strain became apparent due to death of the affected plants.

There is also evidence that strains similar to CPMV-TN arise by spontaneous mutation from the CPMV-type strain Sb during maintenance on live plants in greenhouse, and are detected when the inoculations are made in NLLR-type cowpea cultivar Early Red (C. P. De Jager, *personal communication*).

Based on the above information, the R-, S-, NLLR-, and LS-reacting parents represent the following genotypes (only one allele of each gene is mentioned): *mvs mvn* (TVu 227, -612, -1948, -2331, -2480, -4536, -4539, -4540, -4544, and -6666); *mvs MVN*-1 (TVu 1190, TVx 1836-348, and TVx 1836-481); *mvs MVN*-3 (TVu 317, -345); *MVN*-1 *MVS*(SVS-3, TVu 201, and TVu 222); *MVN*-2 *MVS* (TVu 266-1, -408, and -410); *MVN*-3 *MVS* (TVu 1564-P_2); *mvn MVS* (5/8/2/2, Prima, TVu 2616, CP/2, 1/2/3, P_{33}-1C, and P_{33}-2B).

The results confirm and provide a genetic basis for Robertson's (11) findings that the chlorotic spots, necrotic spots, or no visible symptoms produced on the primary leaves of a cowpea cultivar inoculated with CPMV was related to the cultivar's susceptibility, resistance, or immunity, respectively, to this virus. Lines producing necrotic local lesions in the primary leaves did not develop systemic symptoms in the trifoliolate leaves. However, Williams (16) and Beier et al (2) have reported that some cowpea lines inoculated with CPMV produced necrotic local lesions in primary leaves followed by systemic mosaic. This suggests differences in the CPMV strains and existence of genetic systems in the host different from the one I report here. Earlier, I had found that the cowpea lines reported to be immune, resistant, or predominantly resistant in tests by workers in Nigeria (11,15,16) and the USA (2), produced NLLR reaction to CPMV-LL and LS reaction to CPMV-TN (8). Robertson (11) found that some cowpea lines gave immune reaction to one strain, but produced necrotic local lesions to others. The genetics of the immune reaction to Nigerian and other CPMV strains has not been reported; but, in the system I describe here, the homozygous recessive allele of the *MVS* gene is epistatic to the necrosis gene *MVN* and cannot be used to explain the type of differential reaction Robertson (11) reported. Detailed comparative studies are needed on the available CPMV strains and the differentially resistant lines to establish the genetic relationships and interactions like those done by Drijfhout (7) in bean-bean common mosaic virus, to develop a better basis for breeding cowpea cultivars resistant to CPMV.

The immediate implications of the present genetic studies are that the cowpea disease resistance breeding programs for the areas in East Africa, where both CPMV-LL and CPMV-TN strains might be widely prevalent, should use parents carrying recessive

gene, *mvs mvs*, resistance and screen more cowpea lines to identify additional recessive genes which may be useful when new strains of this virus capable to overcome *mvs mvs* gene are encountered.

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