Identification of a Gene for Resistance to Wheat Streak Mosaic Virus in Maize

Michael D. McMullen and Raymond Louie

United States Department of Agriculture, Agricultural Research Service (USDA-ARS) and the Department of Agronomy, and USDA-ARS and the Department of Plant Pathology, respectively, Ohio Agricultural Research and Development Center-The Ohio State University (OSU-OARDC), Wooster 44691.

We acknowledge the technical assistance of John Abt, Mark Jones, Brenda Schult, and Nasser Abou-Assaf. We thank Don Gordon, The Ohio State University, for antisera to MDMV-A and MDMV-B; Roy French, USDA-ARS, University of Nebraska, for antiserum to WSMV; and Ronald L. Phillips, University of Minnesota, for providing seed of the *po* lines.

This was a cooperative investigation of the USDA-ARS and OSU-OARDC and was submitted as Journal article 295-89 by OSU-OARDC. Salaries and research support were provided by federal and state funds to the USDA-ARS and OSU-OARDC.

Mention of trademark or proprietary products does not constitute a guarantee or warranty of the product by the USDA or OSU-OARDC, and also does not imply approval to the exclusion of other products that may also be suitable.

Accepted for publication 7 January 1991 (submitted for electronic processing).

ABSTRACT

McMullen, M. D., and Louie, R. 1991. Identification of a gene for resistance to wheat streak mosaic virus in maize. Phytopathology 81:624-627.

Wheat streak mosaic virus (WSMV) induces generalized mosaic symptoms in selected maize inbreds. During 1988 and 1989, WSMV was detected in many lines in our maize nursery. WSMV symptoms were associated with the expression of the polymitotic (*po*) marker in a B73 genetic background. The polymitotic locus is on the short arm of maize chromosome 6. An isolate of WSMV (WSMV-W) from naturally infected plants was used to rub-inoculate greenhouse-grown maize plants segregating (*po*/*po* or *po*/+)B73, and the symptom responses of these plants confirmed the presence of a gene linked to *po* that controlled resistance to WSMV. Restriction fragment length polymorphism (RFLP) analysis located this gene on either the short arm of chromosome 6 or on the long arm proximal to the RFLP marker locus UMC59. The symptom responses to inoculation with WSMV were also determined for F_2 and backcross plants from crosses between the WSMV-resistant inbred Pa405 and the WSMV-susceptible inbred Oh28. The segregation ratios suggested the presence of multiple genes for resistance to WSMV in Pa405. RFLP analysis of plants from these crosses demonstrated that one gene for resistance in Pa405 was also located on chromosome 6.

Additional keywords: maize dwarf mosaic virus, potyvirus.

Wheat streak mosaic virus (WSMV), a member of the mite-transmitted subgroup of potyviruses, causes a serious disease in wheat (*Triticum aestivum* L.) (1,2,10). The presence of WSMV in maize in fields adjacent to winter wheat has lead to speculation that maize may serve as an important oversummering host for WSMV and its mite (*Eriophyes tulipae* Keifer) vector (2). Yield losses from wheat streak mosaic cost U.S. wheat producers millions of dollars per year (2,10). Because there are no immune or totally resistant varieties of wheat, considerable effort has been made to introduce WSMV resistance into bread wheat from resistant wheat relatives such as the *Agropyron* species (17).

In addition to infecting wheat, oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.), WSMV infects certain varieties of maize (*Zea mays* L.) (6,11). Although most inbred lines of maize are susceptible to maize dwarf mosaic virus, a member of the aphid-transmitted subgroup of potyviruses, relatively few lines of maize show mosaic symptoms upon inoculation with WSMV (R. Louie and M. D. McMullen, *unpublished*). In this paper, we examine the genetic basis of resistance to WSMV in maize.

MATERIALS AND METHODS

The (*po*/*po* or *po*/+)B73 line was developed by six generations of backcrossing *po* from a marker stock into the B73 inbred background (R. L. Phillips, *personal communication*). Plants homozygous for *po* (*po*/*po*) are male-sterile and have a partial reduction in seed set. Plants heterozygous for *po* (*po*/+) show a normal phenotype.

In 1988, 10 (*po*/*po* or *po*/+)B73 seeds were planted in each of two rows to give a total of 18 plants. In 1989, 25 seeds of the same genotype were planted in each of four rows. Because of poor seed quality and poor germination conditions, only 36 plants were obtained. One plant did not develop a tassel. Plants with tassels in which anthers were not extruded from the glumes were classified as male-sterile (*po*/*po*); plants shedding pollen were classified as fertile (*po*/+). The progeny from crosses made with plants of each phenotype confirmed these classifications. In 1988 and 1989, naturally occurring mosaic symptoms were noted on some of the (*po*/*po* or *po*/+)B73 plants. Visual observation, shortly before and again after anthesis, was used to classify plants as either symptomless or mosaic. Field-grown plants with any amount of symptomatic tissue were classified as mosaic.

The isolate of the WSMV, obtained during the summer of 1988, was designated as wheat streak mosaic virus-Wooster (WSMV-W). This isolate was used for the greenhouse inoculation experiments reported in this paper.

Greenhouse-grown (*po*/*po* or *po*/+)B73 plants were evaluated for their symptom responses to rub-inoculation with WSMV-W. Six seeds of the genotype (*po*/*po* or *po*/+)B73, two seeds of the genotype (*po*/*po* or *po*/+) marker stock, and four seeds each of: *po*/*po*B73 × Pa405; B73 × Pa405; Pa405; B68; yM14; K55; Oh28; A188; and B73 were planted in five replications in greenhouse soil beds. The resulting seedlings were rub-inoculated with WSMV-W four times at 2- to 3-day intervals starting at the three-leaf stage as previously described (9). Plants were scored for virus symptoms at approximately weekly intervals until anthesis and were classified as: generalized mosaic (plants with uniform mosaic symptoms on all leaves that emerged 16 days or later after initial inoculation); limited symptoms (plants with limited streaks of mosaic tissue only on upper leaves); or no symptoms. The plants were also scored for male sterility.

To determine the symptom response of plants from crosses between the WSMV-W resistant inbred Pa405 and the WSMV-W

susceptible inbred Oh28, 11 seeds were planted per row for 10 rows of (Pa405 × Oh28)F_2, five rows of (Pa405 × Oh28) × Oh28, two rows of Pa405 × Oh28, and three rows each of Pa405 and Oh28. The rows were placed in random order, five rows per wood flat (30 × 46 × 8 cm), and the resulting seedlings inoculated as described. If 11 seeds germinated, only the first 10 plants per row were inoculated. Plants were scored for mosaic symptoms at 4- to 5-day intervals until no new symptomatic plants were observed. The experiment was repeated once.

The DNA for Southern analysis was isolated from lyophilized tissue by the CTAB procedure of Saghai-Maroof et al (14). The DNA from the (*po*/*po* or *po*/+)B73 plants and inbred controls was digested with the restriction enzyme *Eco*RI, electrophoresed on agarose gels, transferred to nylon membranes (Zetaprobe; Bio-Rad Laboratories, Richmond, CA), and hybridized with random-prime labeled DNA for the restriction fragment length polymorphism (RFLP) probes BNL6.29, UMC59, and UMC21 as previously described (12). The DNA from plants of the Pa405 × Oh28 crosses and inbred controls was digested with the restriction enzyme *Sst*I and hybridized with labeled DNA for the RFLP probe UMC85.

Protein extracts from infected or healthy Oh28 were prepared for western analysis by grinding leaf tissue in 4 ml of TBST (10 mM Tris [pH 8], 150 mM NaCl, 0.05% Tween 20) per gram fresh weight. The homogenate was centrifuged at 10,000 *g* for 10 min, and an aliquot of the supernatant was mixed (1:1) with Laemmli loading buffer. The sample was boiled for 5–10 min, and 50 μl was electrophoresed on a 12.5% polyacrylamide gel (8). The proteins were transferred to Immobilon-P (Millipore Corp., Bedford, MA) membranes with a Transblot cell (Bio-Rad). The membranes were washed twice with TBST and the reactive sites blocked by incubation with 2% bovine serum albumin in TBST overnight. The membranes were then incubated for 1 h with a 1:1,000 dilution of antiserum produced against isolated virions of either MDMV-A, MDMV-B, or WSMV (Nebraska-type strain). The membranes were washed three times with TBST; then, they were incubated for 30 min with a 1:3,000 dilution of commercial goat-anti-rabbit, alkaline phosphatase-conjugated antibody (Bio-Rad). The membranes were washed three times with TBST, and the color reaction developed as described by Mierendorf et al (13).

RESULTS

Mosaic symptoms were observed on some plants in our maize genetic nursery at Wooster, OH, during 1988 and 1989. In greenhouse tests, Oh28 maize and Monon wheat developed systemic, mosaic symptoms when rub-inoculated with sap from symptomatic tissue. Atlas sorghum (*Sorghum bicolor* L.) occasionally developed a few local lesions on the rub-inoculated leaves, but never developed systemic symptoms. These results were consistent with the host range of WSMV (1). The disease agent, from two samples in 1988 and 10 samples in 1989, was confirmed to be WSMV by reaction with antiserum specific to WSMV (Fig. 1). Protein from symptomatic tissue reacted specifically with a major protein of about 46 kD M_r. This result was consistent with the reported size of WSMV capsid protein (3). There was no reaction with antisera to MDMV-A or MDMV-B (Fig. 1).

During both 1988 and 1989, WSMV symptoms were observed in a line segregating either homozygous recessive or heterozygous for the mutation polymitotic (*po*) (4). The presence of mosaic symptoms was associated with the expression of the male-sterile phenotype (Table 1), and suggested that a genetic component of symptom response to WSMV in maize was linked to *po*. The genetic locus for *po* is located on the short arm of maize chromosome 6 (4). The association of WSMV susceptibility with the *po*/*po* genotype was confirmed after rub-inoculation with WSMV-W on (*po*/*po* or *po*/+)B73 plants (Table 2). All 10 male-sterile plants (*po*/*po*) developed generalized mosaic symptoms within 16 days after the first inoculation. The 19 male-fertile plants (*po*/+) had either no symptoms (14 plants), or very limited, delayed symptoms (five plants). Interestingly, no plants of the original polymitotic marker stock, either male sterile or fertile, had any WSMV-induced symptoms. The inbred lines, yM14, K55, A188, and B73, highly susceptible to MDMV-A and MDMV-B, were all resistant to WSMV-W. Only Oh28 was susceptible to WSMV, MDMV-A, and MDMV-B. Oh28 has previously been reported to be very susceptible to WSMV (11).

RFLP analysis was performed on the DNA of the (*po*/*po* or *po*/+)B73 plants to determine if susceptiblility to WSMV was

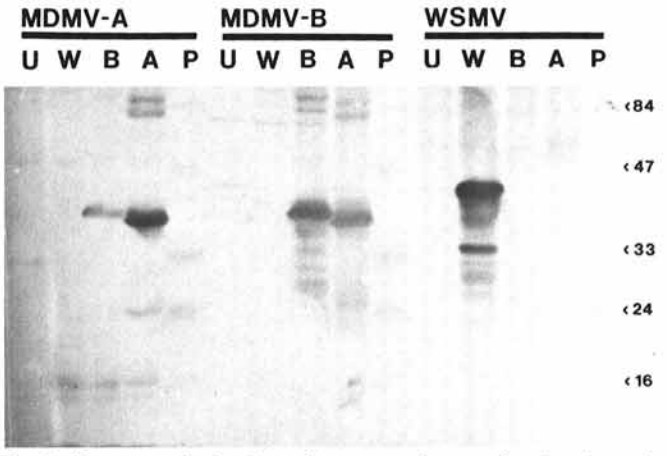

Fig. 1. Western analysis of protein extracts from maize dwarf mosaic virus (MDMV)- and wheat streak mosaic virus (WSMV)-infected tissue. Protein samples: U = uninfected Oh28; W = WSMV-W-infected Oh28; B = MDMV-B-infected Oh28; A = MDMV-A-infected Oh28, plus P = prestained MW markers were incubated with antiserum to MDMV-A, MDMV-B, or WSMV (Nebraska-type strain). Positive serological reactions developed as described in Materials and Methods.

TABLE 1. Symptom response of (*po*/*po* or *po*/+)B73 plants to mosaic virus infection in the field

	Genotype[a]				
	po/+		*po*/*po*		
Year	Mosaic	Healthy	Mosaic	Healthy	Total
1988	0	8	10	0	18
1989	1[b]	18	11	5	35

[a]The number of plants observed either with or without wheat streak mosaic virus-induced symptoms. *po*/+ = male-fertile plants; *po*/*po* = male-sterile plants.
[b]Symptoms were very limited streaks on only the uppermost leaves.

TABLE 2. Symptom responses of maize genotypes of greenhouse-grown plants to rub-inoculation with wheat streak mosaic virus-Wooster (WSMV-W)

	Phenotype[a]						
	Male-fertile			Male-sterile			
Genotype	GM	DL	NS	GM	DL	NS	Total
(*po*/*po* or *po*/+)B73	0	5	14	10	0	0	29
(*po*/*po* or *po*/+)marker stock	0	0	5	0	0	5	10
(*po*/*po*B73 × Pa405)	0	0	20	0	0	0	20
B73 × Pa405	0	0	20	0	0	0	20
Pa405	0	0	15	0	0	0	15
B68	0	0	12	0	0	0	12
yM14	0	0	20	0	0	0	20
K55	0	0	10	0	0	0	10
Oh28	17	0	0	0	0	0	17
A188	0	0	15	0	0	0	15
B73	0	0	19	0	0	0	19

[a]The number of plants with the specific male fertility and symptom responses. GM = plants with generalized mosaic WSMV symptoms; DL = plants with delayed, limited WSMV symptoms; NS = plants with no symptoms.

attributable to a region of chromosome 6 from the *po* stock and to map the extent of the *po* stock chromosome remaining after six backcrosses. All 10 plants with generalized mosaic symptoms and male sterility had only the *po* stock allele at BNL6.29. The 19 symptomless or limited-symptom, male-fertile plants had both *po* stock and B73 alleles at BNL6.29 (Fig. 2). The DNA from all 29 plants had only the recurrent parent (B73) alleles at the RFLP loci UMC59 and UMC21 (data not shown). These results were consistent with the presence of a recessive gene for susceptibility to WSMV located within the segment of DNA from the *po* stock. The corresponding allele for resistance in B73 is located on the short arm of chromosome 6 or the long arm proximal to the RFLP marker locus UMC59 (Fig. 3). We propose to designate this locus as *Wsm1* (wheat streak mosaic virus resistance-gene 1).

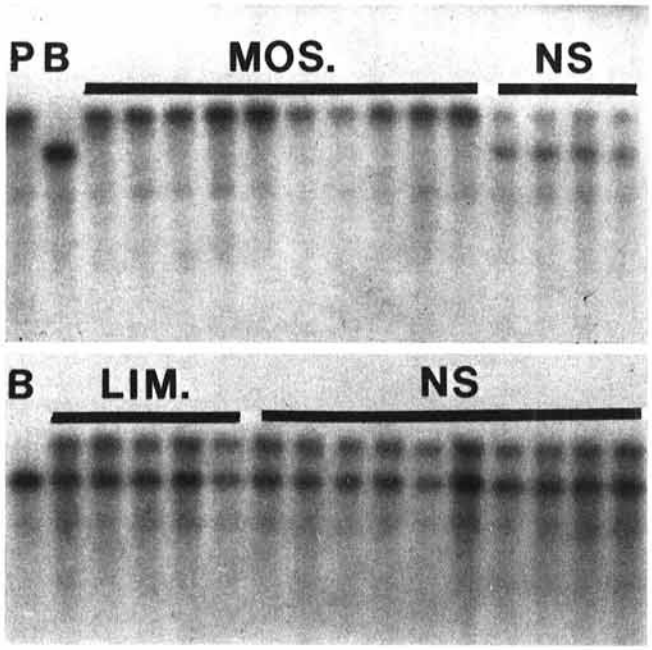

Fig. 2. Southern analysis of DNA from (*po*/*po* or *po*/+)B73 plants with restriction fragment length polymorphism marker probe BNL6.29. The sources of the DNA were: P = *po* stock; B = B73; MOS = (*po*/*po* or *po*/+)B73 plants with generalized mosaic symptoms; LIM = (*po*/*po* or *po*/+)B73 plants with limited, delayed symptoms; NS = (*po*/*po* or *po*/+)B73 plants without symptoms. Southern analysis was done as described in Materials and Methods.

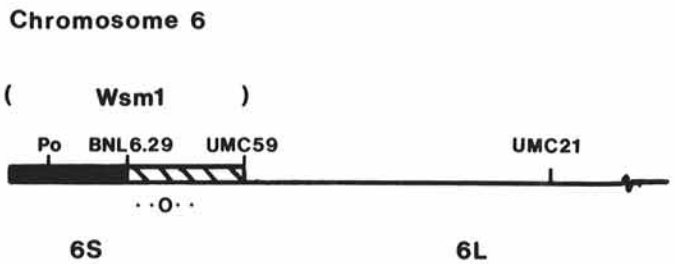

Fig. 3. Map of maize chromosome 6. The solid bar indicates the portion of chromosome 6 from the donor *po* marker stock. The hatched bar indicates the region where the limits of the donor sequence occur. The parentheses indicate the limits of the region that contains *Wsm1*. -O- Indicates the position of the centromere.

The association of WSMV susceptibility with the polymitotic phenotype might be explained by homozygous *po* conditioning susceptibility to WSMV. To test this hypothesis, F_2 and backcross plants of crosses between the resistant inbred Pa405 and the susceptible inbred Oh28 were inoculated with WSMV-W, and their symptom response was observed to determine if susceptibility to WSMV in plants from this cross was also conditioned by a gene on chromosome 6, independent of *po* phenotype. In contrast to the results with the (*po*/*po* or *po*/+)B73 plants, the symptom responses of the F_2 and the backcross plants were not consistent with the hypothesis that a single gene controlled resistance to WSMV (Table 3). In experiment 1, the results from both the F_2 and backcross populations were consistant with two independent genes in Pa405 controlling resistance to WSMV. In experiment 2, the results from the backcross population were consistent with a two-gene model, but the results from the F_2 population were not. To determine if one of the genetic components determining resistance/susceptibility to WSMV in Pa405 was located on chromosome 6, DNA from all the backcross plants and the susceptible F_2 plants was analzyed for the RFLP alleles at the marker locus UMC85. The locus for UMC85 is 1 cM distal to BNL6.29 (12). All 16 backcross plants susceptible to WSMV were homozygous for the Oh28 allele at UMC85 (Table 4). In addition, all nine susceptible F_2 plants were also homozygous for the Oh28 allele at UMC85 (data not shown). Therefore, a requirement for WSMV susceptibility in these plants was that the short arm of both chromosome-6 homologues came from the susceptible Oh28 parent. These results indicated that one of the genetic components for WSMV resistance in Pa405 was located on maize chromosome 6, probably on the short arm, tightly linked to the RFLP marker locus UMC85. For (*po*/*po* or *po*/+)B73, (Pa405 × Oh28)F_2, and (Pa405 × Oh28) × Oh28 plants, a gene or genes on the short arm of chromosome 6 were involved in resistance to WSMV in maize.

DISCUSSION

WSMV susceptibility behaved as a single recessive locus, tightly linked to *po* in the (*po*/*po* or *po*/+)B73 plants. However, when the parental lines were examined, neither was susceptible to WSMV. Because all plants of the original *po* marker stock, and all plants of the recurrent B73 parent were resistant, transgressive segregation (the appearance of a genotype not present in either parent) of WSMV susceptibility may have occurred during the backcrossing of the *po* into B73. The possible presence of multiple,

TABLE 3. Symptom response of (Pa405 × Oh28)F_2 and (Pa405 × Oh28) × Oh28 plants to inoculation with wheat streak mosaic virus-Wooster (WSMV-W)[a]

		Experiment 1[d]					Experiment 2				
		Resistant		Susceptible			Resistant		Susceptible		
Genotype[b]	R/S[c]	Observed	Expected	Observed	Expected	*P*	Observed	Expected	Observed	Expected	*P*
(P × O)F_2	15R/1S	91	93	8	6	0.5–0.3	93	88	1	6	0.05–0.02
(P × O) × O	3R/1S	42	37.5	8	12.5	0.2–0.1	42	37.5	8	12.5	0.2–0.1
P × O	100%R	20	20	0	0	···	20	20	0	0	···
Pa405	100%R	20	20	0	0	···	23	23	0	0	···
Oh28	100%S	0	0	30	30	···	0	0	30	30	···

[a]The number of plants with the symptom responses indicated.
[b](P × O)F_2 = (Pa405 × Oh28)F_2; (P × O) × O = (Pa405 × Oh28) × Oh28; P × O = Pa405 × Oh28.
[c]Ratio of resistant to susceptible plants expected for two dominant, independent genes for resistance in Pa405.
[d]Number of resistant plants with no symptoms and susceptible plants with systemic WSMV-induced mosaic symptoms. *P* = chi-square probability.

TABLE 4. Association of symptom response of (Pa405 × Oh28) × Oh28 plants to wheat streak mosaic virus-Wooster (WSMV-W) with restriction fragment length polymorphism alleles at UMC85[a]

Genotype at UMC85	R/S[b]	Number of plants[c]				
		Resistant		Susceptible		
		Observed	Expected	Observed	Expected	*P*
Pa405/Oh28	100% R.	61	61	0	0	...
Oh28/Oh28	1R/1S	22	19	16	19	0.5–0.3

[a]Number of either resistant or susceptible plants for the genotype indicated.
[b]Ratio of resistant to susceptible plants expected for two dominant, independent genes for resistance in Pa405.
[c]Resistant plants had no symptoms; susceptible plants showed systemic WSMV-induced mosaic symptoms. *P* = chi-square probability.

independent genes for resistance to WSMV may explain the lack of susceptibililty in the parental lines of the *po* B73 plants. The inbred B73 may be resistant to WSMV because it has a resistant allele of the gene on chromosome 6. The original *po* stock plant apparently had a susceptible allele on chromosome 6, but may have a second independent gene for WSMV resistance. Backcrossing eliminated that second resistance gene present in the *po* stock allowing resistance or susceptibility to be determined by the alleles on chromosome 6. An alternative explanation for the lack of susceptibility of the parental lines is that a mutation to susceptibility occurred during the backcross program.

The results from the analysis of symptom response of the progeny of the Pa405 × Oh28 cross was not consistent with a single gene controlling WSMV susceptibility. The backcross data and one test of the F_2 population could be explained by requiring two independent recessive genes from Oh28 for WSMV susceptibility. Further genetic analysis will be required to confirm the presence of a second gene for susceptibility in Oh28. The identification of multiple loci based on segregation ratios alone is always suspect because of the difficulties in separating primary genetic effects from genotype × environment interactions. Our RFLP data indicated that one of the required genetic components of susceptibility is located on maize chromosome 6.

We have previously identified a major gene for resistance to MDMV-A on the short arm of maize chromosome 6 (12). The chromosomal region that contains *Wsm1* also includes this MDMV resistance gene, *Mdm1*. These results raise the possibility of allelism between *Mdm1* and *Wsm1*. Single genes, pleiotropic for resistance to more than one plant virus are rare and to date have been described only for viruses within the aphid-transmitted subgroup of potyviruses (7,16). If *Wsm1* and *Mdm1* are allelic, our results would identify the most divergent viruses yet determined to be controlled by a single genetic locus. Alternatively, *Wsm1* and *Mdm1* may simply be closely linked loci controlling resistance to related pathogens as has been described for genes on the short arm of chromosome 10 controlling resistance to different species of maize rust (5,15). Regardless of whether the genes, *Mdm1* and *Wsm1*, are allelic or closely linked, selection for the short arm of chromosome 6 from a line resistant to both viruses, such as Pa405, should permit selection for resistance to both pathogens.

LITERATURE CITED

1. Brakke, M. K. 1971. Wheat streak mosaic virus. No. 48 in Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol. Kew, Surrey, England.
2. Brakke, M. K. 1987. Virus diseases in wheat. Pages 585-603 in: Wheat and Wheat Improvement. 2nd ed. E. G. Heyne, ed. ASA-CSSA-SSSA, Madison. 765 pp.
3. Brakke, M. K., Ball, E. M., Hsu, Y. H., and Langenberg, W. G. 1987. Wheat streak mosaic virus cylindrical inclusion body protein. J. Gen. Virol. 68:281-287.
4. Coe, E. H., Neuffer, M. G., and Hoisington, D. A. 1988. The genetics of corn. Pages 81-258 in: Corn and Corn Improvement. 3rd ed. G. F. Sprague and J. W. Dudley, eds. ASA-CSSA-SSSA, Madison. 986 pp.
5. Hooker, A. L., and Saxena, K. M. S. 1971. Genetics of disease resistance in plants. Annu. Rev. Genet. 5:407-424.
6. How, S. C. 1963. Wheat streak mosaic virus on corn in Nebraska. Phytopathology 53:279-280.
7. Kyle, M. M., and Dickson, M. H. 1988. Linkage of hypersensitivity to five Viruses with the *B* locus in *Phaseolus vulgaris* L. J. Hered. 79:308-311.
8. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) 227:680-685.
9. Louie, R. 1986. Effects of genotype and inoculation protocols on resistance evaluation of maize to maize dwarf mosaic virus strains. Phytopathology 76:769-773.
10. McKinney, H. H. 1967. Viruses diseases. Pages 355-374 in: Wheat and Wheat Improvement. 1st ed. K. S. Quisenberry and L. P. Reitz, eds. ASA-CSSA-SSSA, Madison. 560 pp.
11. McKinney, H. H., Brakke, M. K., Ball, E. M., and Staples, R. 1966. Wheat streak mosaic virus in the Ohio valley. Plant Dis. Rep. 50:951-953.
12. McMullen, M. D., and Louie, R. 1989. The linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. Mol. Plant-Microbe Interact. 2:309-314.
13. Mierendorf, R. C., Percy, C., and Young, R. A. Gene isolation by screening lambda-*gt11* libraries with antibodies. Methods Enzym. 152:458-469.
14. Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, R. A., and Allard, R. W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.
15. Saxena, K. M. S., and Hooker, A. L. 1968. On the structure of a gene for disease resistance in maize. Proc. Natl. Acad. Sci. USA 61:1300-1305.
16. Schroeder, W. T., and Provvidenti, R. 1971. A common gene for resistance to bean yellow mosaic virus and watermelon mosaic virus 2 in *Pisum sativum*. Phytopathology 61:846-848.
17. Stoddard, S. L., Gill, B. S., and Lommel, S. A. 1987. Genetic expression of wheat streak mosaic virus resistance in two wheat-wheatgrass hybrids. Crop Sci. 27:514-519.