Three Genetic Loci Control Resistance to Wheat Streak Mosaic Virus in the Maize Inbred Pa405

Michael D. McMullen,[1,2] Mark W. Jones,[1,2] Kevin D. Simcox,[1] and Raymond Louie[1,3]

[1]Corn & Soybean Res. Unit, USDA-Agricultural Research Service, OARDC, Wooster, OH 44691 U.S.A.; [2]Dept. of Agronomy, OARDC, Wooster, OH 44691, U.S.A.; [3]Dept. of Plant Pathology, OARDC, Wooster, OH 44691, U.S.A.

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Wheat streak mosaic virus (WSMV), a mite-transmitted potyvirus, infects certain maize inbreds. To identify genes for resistance to WSMV, (Pa405 × Oh28)F_2 plants were inoculated with WSMV and symptom responses observed. In addition to resistant (symptomless) plants, two types of symptomatic plants were noted: plants with generalized mosaic (GM) similar to the symptoms observed on the susceptible inbred Oh28, and plants with dispersed, chlorotic spots and rings (DSR). DNAs pooled from 25 plants with GM symptoms and from 25 plants with DSR symptoms were used to detect linkage to RFLP loci by "bulked segregant" analysis. This analysis identified two previously unreported genes for resistance to WSMV, designated *wsm2* (chromosome 3 near *umc102*) and *wsm3* (chromosome 10 near *umc163*), and confirmed the presence of *wsm1* on chromosome 6S. RFLP analyses of DNA from individual plants revealed that the plants that exhibited GM symptoms were homozygous for Oh28 alleles at *wsm1*, *wsm2*, and *wsm3*. Plants that exhibited DSR symptoms were homozygous for Oh28 alleles at *wsm1* and *wsm2*, but were heterozygous or homozygous for the allele from Pa405 at *wsm3*.

Wheat streak mosaic virus (WSMV), a member of the mite-transmitted subgroup of potyviruses, causes a major disease of small grains (Brakke 1971). WSMV also infects certain varieties of maize. Although most maize inbred lines are susceptible to maize dwarf mosaic virus (MDMV), a member of the aphid-transmitted subgroup of potyviruses, relatively few lines of maize show mosaic symptoms upon inoculation with WSMV (McMullen and Louie 1991, unpublished observations). This is not due to a general inability of WSMV to induce symptoms in maize as symptoms on inbreds susceptible to both WSMV and MDMV are more severe upon infection with WSMV (McMullen and Louie, unpublished results). McMullen and Louie (1991) identified a gene (*wsm1*) for resistance to WSMV on chromosome 6S of B73 and Pa405. Analysis of segregation ratios of resistant to susceptible plants in F_2 and backcross progeny from the cross Pa405 × Oh28 indicated that additional genetic factors besides *wsm1* in Pa405 control symptom response to WSMV. To identify the additional genetic loci controlling resistance to WSMV, we have performed RFLP analysis using DNA pools (Michelmore *et al.* 1991) of two classes of susceptible (Pa405 × Oh28)F_2 individuals. This analysis identified two additional genes in Pa405 for resistance to WSMV, designated *wsm2* (chromosome 3 near *umc102*) and *wsm3* (chromosome 10 near *umc163*), and confirmed the presence of *wsm1* on chromosome 6S.

Current address of M. D. McMullen and K D. Simcox: USDA ARS, Curtis Hall, University of Missouri, Columbia, MO 65211, U.S.A.

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RESULTS

The reaction of maize inbred Pa405 to inoculation with WSMV was a symptomless response. The virus was detected in inoculated leaves by either bioassay or ELISA techniques (McMullen and Louie 1991, unpublished data). The maize inbred Oh28 exhibited pronounced WSMV-induced symptoms. These symptoms began with an acute initial phase where chlorotic spots coalesced and became almost completely white (these symptoms were present on about two leaves) followed by a bright yellow-green mosaic on all subsequently emerging leaves.

For the first screening, 12 out of 1,100 (Pa405 × Oh28)F_2 plants exhibited clear generalized mosaic (GM) symptoms. However, only 70% of the susceptible parental Oh28 plants were symptomatic. The cause for the low rate of symptom expression of Oh28 plants is unknown.

The rub-inoculation method (Louie 1986) was used for the second set of seedlings and all plants of the susceptible inbred Oh28 exhibited GM symptoms. Twenty-two out of 1,100 (Pa405 × Oh28)F_2 plants exhibited GM symptoms that were similar in timing of appearance and severity to the susceptible Oh28 plants. The (Pa405 × Oh28)F_2 plants were allowed to continue to grow and 27 additional plants exhibited a second, distinct class of WSMV-induced symptoms that consisted of dispersed, chlorotic spots and rings (DSR). The appearance of symptoms on these plants was delayed relative to the appearance of GM symptoms and the symptoms affected a limited leaf area.

The simplest genetic model that explains the low number of infected F_2 plants is the presence of multiple genes in Pa405 that are individually sufficient to confer resistance to WSMV. If this is the correct model then there are a large number of genotypes in the symptomless (Pa405 × Oh28)F_2 plants, but limited genotypes for the symptomatic plants. That is, symptomatic plants must lack Pa405 alleles at multiple

loci. An efficient approach to test the model and map the resistance loci would be to conduct "interval mapping" (Hoisington and Coe 1989) on pools of DNA from susceptible plants (Michelmore *et al.* 1991; Churchill *et al.* 1993).

To identify the general chromosomal regions affecting resistance Southern hybridization analyses were conducted on pooled DNAs from plants of the GM and DSR symptom classes hybridized with the maize "Core" RFLP markers (see Materials and Methods). The hybridization pattern of *bnl5.62* was indicative of a chromosomal region not involved in resistance since there was an approximately equal representation of Pa405 and Oh28 alleles in both the GM and DSR pools (Fig. 1A). On the maize genetic map *bnl5.62* is located on chromosome 1-position 0 (Maize Genetics Cooperation Newsletter Vol. 69, 1993). We defined an equal representation of alleles as "unbiased" and a predominance of one allele as "biased" (indicating linkage to resistance). Three separate chromosomal regions detect by the probes p-umc102, chromosome 3-position 78 (Fig. 1B), p-umc85, chromosome 6-position 2 (Fig. 1C), and p-umc44, chromosome 10-position 122 (Fig. 1D) gave hybridization patterns consistent with linkage to genetic factors for resistance to WSMV. Hybridization with p-umc102 gave an almost complete bias to the Oh28 allele in both the GM and DSR pools, indicating the presence of a dominant gene for resistance to WSMV on chromosome 3 in Pa405. We designated this gene *wsm2*. Hybridization with p-umc85 also revealed an almost complete bias to the Oh28 allele in both the GM and DSR pools. This result confirmed the previous report (McMullen and Louie 1991) of a dominant gene (*wsm1*) for resistance to WSMV on chromosome 6S from Pa405. The genetic basis of the difference between plants with GM compared to DSR symptoms was revealed with the RFLP probe p-umc44. While there was an almost complete bias to the Oh28 allele in the GM pool, there was almost equal representation of Pa405 and Oh28 alleles in the DSR pool. This result indicated that there was a gene on chromosome 10 of Pa405, which we designated *wsm3*, that will give partial seedling resistance to WSMV. These results indicated that plants in the GM pool were of the genotype *wsm1/wsm1*, *wsm2/wsm2*, *wsm3/wsm3*; plants in the DSR pool were of the genotype *wsm1/wsm1*, *wsm2/wsm2*, *Wsm3/-*. The extent of coverage of the maize genome with polymorphic RFLP loci is shown in Figure 2.

The bulked segregate analysis indicated that genetic loci near the centromere of chromosome 3 (*wsm2*), the short arm of chromosome 6 (*wsm1*), and the long arm of chromosome

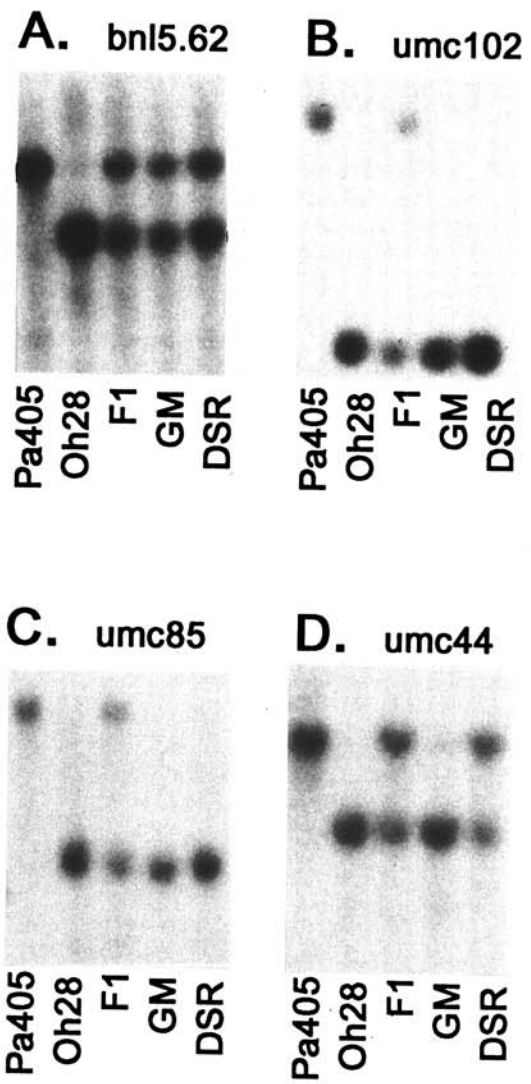

Fig. 1. Southern hybridization analysis for mapping genes for resistance to WSMV. DNAs from Pa405, Oh28, F_1, pool of 25 (Pa405 X Oh28)F_2 plants with generalized mosaic symptoms (GM), and pool of 25 (Pa405 X Oh28)F_2 plants with dispersed, chlorotic spots and rings (DSR) symptoms were digested with restriction enzymes, transferred to membranes and hybridized with the public "core" probes (Gardiner *et al.* 1993) as described in Materials and Methods. Shown are informative probe/enzyme combinations for four RFLP probes, **A,** p-bnl5.62-*Bam*HI, detects *bnl5.62,* Ch1-0 (chromosome #-map position); **B,** p-umc102-*Hind*III, detects *umc102,* Ch3-71; **C,** p-umc85-*Eco*RV detects *umc85,* Ch6-2; and **D,** p-umc44-*Hind*III, detects *umc44,* Ch10-122.

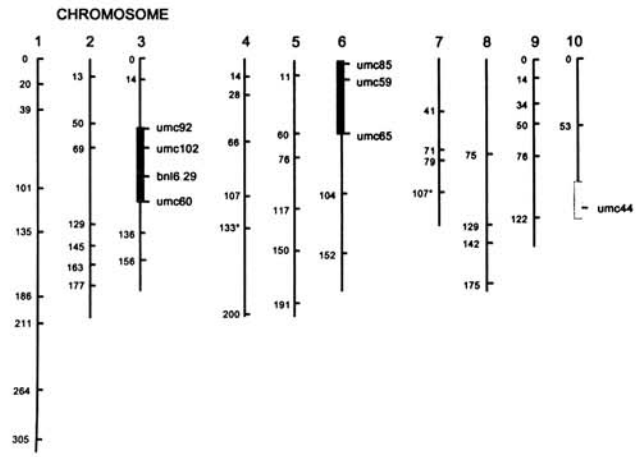

Fig. 2. Genetic map of chromosomal regions tested for association with resistance to WSMV in Pa405. The loci indicated by a map position only are "core" loci (Gardiner *et al.* 1993) that exhibited an unbiased (equal) distribution of Pa405 and Oh28 alleles in both the GM and DSR pools. The positions with an * indicate additional loci (*umc15*, Ch 4-133; *bnl12.06*, Ch 7-107) that exhibited an unbiased distribution. The core RFLP loci associated with the cross-hatched regions of chromosomes 3 and 6 were biased for Oh28 alleles for both the GM and DSR pools. The core RFLP locus *umc44* associated with the stippled region on chromosome 10 was biased for Oh28 alleles in the GM pool and unbiased in the DSR pool (see Fig. 1).

10 (*wsm3*) control resistance to WSMV. To obtain more precise map positions for these loci the DNAs of a total of 61 individual F_2 plants with either GM (34 plants) or DSR (27 plants) symptoms were typed for RFLP alleles at loci within chromosomal regions linked to resistance. RFLP allele data for all 61 plants were used for each of the three genes. The map positions of *wsm1, wsm2,* and *wsm3* relative to linked RFLP markers were determined using MAPMAKER (Lander *et al.* 1987) as described in Materials and Methods. The map positions of *wsm1, wsm2,* and *wsm3* are shown in Figure 3.

DISCUSSION

In this study, bulked segregant analysis was successful in identifying three genes for resistance to WSMV in Pa405. Three classes of symptoms were observed in a segregating F_2 population and the inheritance of the symptom classes explained by the three genetic loci mapped in this study, *wsm1, wsm2,* and *wsm3*. The use of pooled DNA samples from the susceptible classes to identify and map these loci was much more efficient than the standard practice of mapping all individuals in a segregating F_2 population. This approach should be useful for defining the genetic basis of any trait that is expressed by a specific and infrequent genotype in a segregating population.

The genotype of plants with GM symptoms was determined to be *wsm1/wsm1*, *wsm2/wsm2*, and *wsm3/wsm3*. The genotype of DSR plants was determined to be *wsm1/wsm1, wsm2/wsm2,* and *Wsm3/-*. In an F_2, the genotype for the GM class should occur in 1/64 individuals and the genotype of the DSR plants in 3/64 individuals. In the second experiment (1,100 F_2 plants) there were 22 plants with GM symptoms (17 expected) and 27 plants with DSR symptoms (51 expected). While chi-square tests reveal that the number of plants expressing GM symptoms was consistent with the model ($0.3 < P < 0.2$), the number of plants expressing DSR symptoms was less than predicted ($0.001 < P$). The DSR phenotype is a very limited symptom expression type. It may be that some individuals with a genotype for DSR may have remained symptomless through the duration of the experiment. This apparent lack of penetrance of the DSR phenotype may have environmental or developmental bases. Although this suggests that there are a limited number of misclassified individuals in the symptomless class (24 additional expected out of 1,059) this would not affect the conclusions of this study as all mapping was performed with individuals from the GM and DSR classes.

wsm1 was located on the short arm of chromosome 6 between the RFLP loci *umc85* and *npi235* near the nucleolus organizer region (NOR). This is in the same region of chromosome 6S as *mdm1*, a major gene for resistance to MDMV (McMullen and Louie 1989, unpublished data). While the particle morphology, cytopathology, and the mosaic symptom pattern are similar between WSMV and the aphid-transmitted potyvirus MDMV, the differences in vectors and capsid protein sequences (Niblett *et al.* 1991) have led to the establishment of a separate genus, the rymoviruses, for WSMV and related mite-transmitted potyviruses (Barnett 1991). It is not known if *mdm1* and *wsm1* are allelic; i.e., whether the dominant resistance alleles *Mdm1* and *Wsm1* are the same gene. Single-gene resistance for viruses is usually very specific (Fraser 1990), and the potential allelism between *wsm1* and *mdm1* may involve host interaction with a conserved property of potyvirus multiplication. A third gene for resistance to a major maize pathogen, *Cochliobolus heterostrophus* race O, the pathogen for southern corn leaf blight, also has been localized to this region. RFLP analysis has been used to map the *rhm1* gene, which confers a recessive genetic resistance to *C. heterostrophus* race O, to the short arm of chromosome 6 very close to *umc85* (Zaitlin *et al.* 1993).

wsm2 was located near the centromere of chromosome 3 between the RFLP marker loci *umc102* and *umc18*. This map position placed *wsm2* close to the position of *rp3*, a dominant gene for resistance to *Puccinia sorghi*, the pathogen for

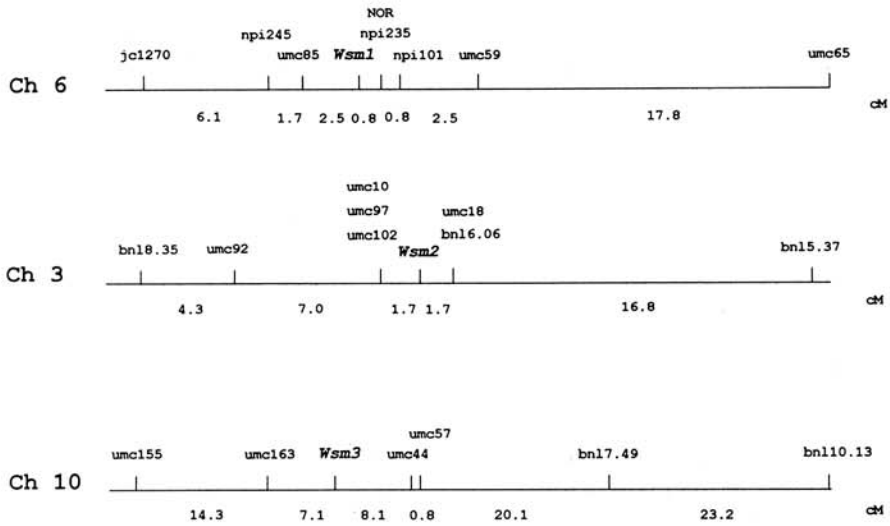

Fig. 3. The map positions of *wsm1, wsm2,* and *wsm3*. Thirty-four individuals with GM symptoms and 27 individuals with DSR symptoms were typed for RFLP alleles within the regions of chromosomes 3, 6, and 10 that were biased on the interval screening hybridizations. All individuals in the GM pool were assigned the genotype *wsm1/wsm1, wsm2/ wsm2, wsm3/wsm3* (all recessives from Oh28), individuals in the DSR pool were assigned the genotype *wsm1/wsm1, wsm2/wsm2, Wsm3/–*. The loci order and map distances were determined using MAPMAKER, version 3.0 for DOS.

common corn rust, again suggesting a possible clustering of genes for resistance to maize pathogens.

wsm3 was located on the long arm of chromosome 10 between the RFLP marker loci *umc163* and *umc44*. This is the first report of a disease resistance gene on the long arm of maize chromosome ten.

It is interesting to compare the genetic basis of resistance in Pa405 to MDMV (McMullen and Louie 1989; Louie *et al.* 1990) with resistance in Pa405 to WSMV (McMullen and Louie 1991, this paper). For resistance to MDMV, the dominant allele *Mdm1* is required for any level of seedling resistance. Upon inoculation with MDMV all plants without *Mdm1* rapidly develop GM symptoms. In contrast, for resistance to WSMV each of the alleles *Wsm1, Wsm2,* or *Wsm3* appears sufficient to reduce symptom expression in maize seedlings. Resistance to MDMV in all inbreds examined to date has involved a gene on chromosome 6, presumably *mdm1* (McMullen and Louie 1989; Roane *et al.* 1989; McMullen and Louie, unpublished data). The potential presence of multiple resistance genes may explain why so few maize inbreds are susceptible to WSMV.

WSMV is adapted primarily to small grains, and no known varietal resistance occurs within bread wheat. Because of the widespread resistance in maize inbreds, WSMV appears to be only secondarily adapted to maize. This paper documents three loci in the maize inbred Pa405 that restrict WSMV-induced symptom expression. We are developing near isogenic lines with *Wsm1, Wsm2,* and *Wsm3* to study the biological basis of resistance encoded by these genes.

MATERIALS AND METHODS

WSMV inoculation.

The Wooster isolate of WSMV was used in all experiments (McMullen and Louie 1991).

Eleven-hundred (Pa405 × Oh28)F_2 kernels were planted in flats, along with parental and F_1 controls in each of two screenings. For the first screening, plants were inoculated with WSMV (Wooster isolate) three times at 2- to 3-day intervals starting at the two- to three-leaf stage using an artist airbrush. Plants were observed at 2- to 3-day intervals for WSMV-induced symptoms.

For the second screening 1,104 (Pa405 × Oh28)F_2 kernels were planted in flats, along with parental and F_1 controls, and the seedlings were rub-inoculated 4 times at 2- to 3-day intervals starting at the two- to three-leaf stage (Louie 1986).

Interval mapping and RFLP analysis.

DNA was extracted from lyophilized, ground tissue by the CTAB procedure (Saghai-Maroof *et al.* 1986). The GM pool of DNA consisted of equal amounts of DNA from 10 plants with generalized mosaic (GM) symptoms from the first screening and 15 plants with GM symptoms from the second screening. The DSR pool of DNA consisted of equal amounts of DNA from 25 plants with dispersed, chlorotic spots and rings symptoms (DSR) (see results for description) from the second screening. Ten micrograms of DNA each from Pa405, Oh28, F_1, GM pool, and DSR pool were digested with *Eco*RI, *Bam*HI, and *Hin*dIII and electrophoresed on 0.8% agarose gels. The DNA was then transferred to Genescreen-Plus hybridization membrane (Dupont-NEN, Boston, MA) by our modification (Simcox and McMullen, see Maize Genetics Coop. Newsletter 67:116-117) of the "dry blot" procedure (Kempter *et al.* 1991). The membranes were prehybridized (5 × SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 2× Denhardt's, 50 mM Tris, pH 8.0, 5 mM Na_2EDTA, 0.5% SDS, 250 µg/ml denatured salmon sperm DNA) for 3–4 hr at 65° C. Radioactive probes were prepared from isolated inserts by random priming (Feinberg and Vogelstein 1983). The membranes were hybridized with probes in a solution of the same constituents as above plus 10% dextran sulfate. The BNL and UMC RFLP probes of the "core probe set" (Gardiner *et al.* 1993) were used. The "core probe set" consists of 97 RFLP markers that were chosen to give maximum coverage of the maize genome with RFLP probes that exhibited strong signal intensity and high levels of polymorphism among maize lines (Gardiner *et al.* 1993). If a useful polymorphism was not detected with DNAs cleaved with *Eco*RI, *Bam*HI, and *Hin*dIII, an additional filter with DNAs cleaved with the enzymes *Eco*RV, *Dra*I, *Xba*I was tested. In some cases, if core probes did not detect polymorphism between Oh28 and Pa405, additional UMC and/or BNL (Burr *et al.* 1988) RFLP probes with loci reported near the core probe loci were tested. Membranes were washed and films developed as previously described (McMullen and Louie 1989 1991).

Linkage analysis.

Once regions of the genome associated with resistance were identified, the map positions of the corresponding resistance genes were defined by linkage analysis of the alleles present at RFLP loci in the individual symptomatic plants. The dominant alleles for resistance from Pa405 on chromosomes 6, 3, and 10 were designated *Wsm1, Wsm2,* and *Wsm3,* respectively. The recessive susceptible alleles from Oh28 were designated *wsm1, wsm2,* and *wsm3*. For performing linkage analysis the genotype of individual GM plants at resistance loci was defined as *wsm1/wsm1, wsm2/wsm2,* and *wsm3/wsm3*. The genotype of DSR plants was defined as *wsm1/wsm1, wsm2/wsm2,* and *Wsm3/-*. Linkage analysis was performed and map distances determined using MAPMAKER (Lander *et al.* 1987), Version 3.0 for DOS. Confirmation of linkage of marker loci was obtained using the "group" function tested at a LOD of 3.0. An initial map for each region was derived for five RFLP markers using the "compare" function. The positions of the additional RFLP loci and of the resistance genes were added by sequential use of the "try" function.

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