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Co-segregation of the maize dwarf mosaic virus resistance gene, *Mdm1*, with the nucleolus organizer region in maize

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Abstract The *mdm1* locus on the short arm of chromosome six confers resistance in maize to five strains of the *m*aize *d*warf *m*osaic *v*irus (MDMV), an aphid transmitted potyvirus. The location of *mdm1* in relation to RFLP and morphological loci on the short arm of chromosome six was determined using BC_1 and F_2 mapping populations. The following map order and distance in cM was obtained from the F_2 population; *jc1270*-2.5-*npi245*-1.6-*umc85*/*po1*-0.5-*mdm1*/*nor*-0.5-*bnl6.29A*-0.5-*npi235*-0.8-*npi101A*-4.3-*numc59*. No recombination between *mdm1* and the nucleolus organizer region (*nor*) was detected, as determined using a probe from the intergenic spacer region of the rDNA repeat. In order to resolve the relationship between *mdm1* and the *nor*, and to recover recombinants around *mdm1*, a high-resolution map within the *polymitotic1* (*po1*) *yellow kernel1* (*y1*) interval was generated using [*po1 y1* tester (*po1 mdm1 y1*) × Pa405 (*Po1 Mdm1 Y1*)] F_2 plants. The recessive *po1* allele imparts a male-sterile phenotype when homozygous and since *po1* and *y1* are closely linked, the majority of fertile plants from white endosperm (*y1*/*y1*) F_2 kernels will arise though a recombination event between the Pa405 *Po1* allele and the *y1* allele of the *po1 y1* tester. Plants from 7,650 white (*y1*/*y1*) F_2 kernels were examined (15,300 chromosomes) and a total of 626 $F_{2:3}$ recombinant families was recovered. Analysis of these recombinants revealed that *mdm1* cosegregates with the *nor*. This lack of recombination between *mdm1* and the *nor* suggests that: either (1) *mdm1* is located in the region flanking the *nor* and recombination is suppressed within that region, or (2) *mdm1* is located within the *nor*.

Key words Disease resistance · High resolution genetic map · Recombination · Ribosomal DNA · RFLP

Introduction

A major obstacle in elucidating the biochemical basis of disease resistance in plants is the lack of cloned resistance genes. This defficiency was overcome in recent reports involving the cloning of plant disease resistance genes via transposon tagging and chromosome walking (Johal et al. 1992; Martin et al. 1993). To understand the mechanism of resistance in maize to maize dwarf mosaic virus (MDMV), we initiated a chromosome-walking approach to clone the major gene for resistance in maize to MDMV. Maize dwarf mosaic virus is a member of the aphid-transmitted potyviruses. Resistance to five strains of MDMV that infect maize can be attributed to a dominant gene located on the short arm of maize chromosome six (Louie et al. 1991). This gene, *mdm1*, was previously mapped to the interval between the RFLP markers *umc85* and *bnl6.29A* (McMullen and Louie 1989). Resistance to MDMV conferred by the *Mdm1* allele appears to act by restricting long-distance transport of virus particles via the vasculature (Lei and Agrios 1986; Law et al. 1989; McMullen and Louie unpublished data). Cloning of the *Mdm1* allele should allow determination of the biological basis of *Mdm1*-mediated resistance.

Cloning a gene by chromosome walking requires a high-resolution linkage map in the region around the gene of interest (Martin et al. 1993). Furthermore, recovery of individuals with crossovers between the target locus and flanking RFLP markers is a prerequisite for determining the direction and physical distance to be crossed during a chromosome walk. This study describes the development of a high-resolution RFLP map

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in the region of *mdm1* and the association of *mdm1* with the nucleolus organizer region (*nor*) in maize.

Materials and methods

Plant and pathogen sources

Inbreds Pa405 and yM14 were obtained from W. F. Findley, USDA-ARS (retired), Department of Agronomy, OARDC-Ohio State University; *po1* B73 from Ron Phillips, Department of Agronomy and Plant Genetics, University of Minnesota; RB37*rhm1* from Wayne Pedersen, Department of Plant Pathology, University of Illinois at Urbana-Champaign; and the *po1 y1* tester (601G) and the TB-6Sa hyperploid B-A translocation (614B) from the Maize Genetics Stock Center, Department of Agronomy, University of Illinois at Champaign-Urbana. The isolate of *Cochliobolus heterostrophus* race O used to determine *rhm1* genotypes was obtained from Wayne Pedersen. All MDMV inoculations were performed using the Wooster MDMV strain-A type isolate.

Mapping populations

Mapping of *mdm1* with respect to molecular and morphological markers on chromosome 6S was accomplished using BC_1 and F_2 populations. The BC_1 population consisted of the MDMV-resistant yellow endosperm inbred Pa405 (*Mdm1 Y1*) backcrossed by the recurrent parent, yM14, a MDMV-susceptible white endosperm inbred (*mdm1 y1*). Yellow (*Y1/–*) versus white (*y1/y1*) endosperm color is controlled by the *y1* locus, located approximately 5 cM proximal from *mdm1* on chromosome 6L (McMullen and Louie 1989). BC_1 seeds were separated on the basis of endosperm color, planted in a disease nursery, and the plants were screened for susceptibility to MDMV as described below. Tissue for RFLP analysis was collected at about the 12-leaf stage and the BC_1 progeny were self pollinated. Twenty-five kernels from each BC_1 family recombinant between *mdm1* and *y1* were planted in the greenhouse and the seedlings re-screened for MDMV susceptibility. The F_2 population was generated by crossing the MDMV-A-susceptible male-sterile inbred, *po1*B73 (*po1 mdm1/po1 mdm1*), with Pa405 (*Po1 Mdm1/Po1 Mdm1*). Plants homozygous recessive for the *po1* allele exhibit a male-sterile phenotype which appears to be the consequence of multiple rounds of mitosis without DNA replication during pollen development (Phillips 1993). The *po1* locus was previously mapped to chromosome 6S and shown to be located distal to the chromosome 6S breakpoint in TB-6Sa. Cytologically, TB-6Sa appears to split the *nor* heterochromatin region indicating that *po1* maps distal to the *nor*. The *po1*B73 inbred is susceptible to MDMV, therefore the chromosome donor segment containing the recessive *po1* allele is also recessive for *mdm1*. F_2 kernels were planted in a disease nursery and the plants scored for MDMV susceptibility and fertility. Whorl tissue from plants at the 12th leaf stage was collected for RFLP analysis.

The high-resolution mapping population for the *po1*-*y1* interval was generated by crossing a MDMV susceptible, male-sterile, white endosperm, *po1 y1* (*po1 mdm1 y1*) tester with Pa405 (*Po1 Mdm1 Y1*). Selection for recombinants between *y1* and *po1* involved planting the white endosperm F_2 kernels (*y1/y1*) and then identifying fertile F_2 progeny. Crossovers between the Pa405 *Po1* allele and the *y1* allele from the *po1 y1* tester will result in fertile F_2 progeny that carry the dominant *Po1* allele. Fertile F_2 progeny were selfed and 25 seeds from each of the resulting $F_{2:3}$ families were planted in the greenhouse. Leaf tissue from 2-week-old seedlings was bulked for RFLP analysis. Families that exhibited crossovers within the *umc85*-*bnl6.29A* interval were screened in the greenhouse for MDMV susceptibility.

The B-A translocation, TB-6Sa, was used to map molecular markers present on chromosome 6S in relation to the *nor*. The breakpoint on chromosome 6S in TB-6Sa is in the center of the *nor* heterochromatin region (Phillips 1978). Plants hypoploid for the B^6 interchange chromosome will be hemizygous for all loci distal to the breakpoint (Beckett 1982). Hypoploid individuals were generated by crossing a hyperploid TB-6Sa plant onto a RB37*rhm1* tester. The recessive *rhm1* allele, which confers resistance to *C. heterostrophus* race O, has been previously shown to map distal to the TB-6Sa breakpoint (authors, unpublished data). Seeds of F_1 progeny were planted in the greenhouse and the plants screened for resistance to *C. heterostrophus* race O as described below. Leaf tissue was collected at the 10th leaf stage for RFLP analysis.

MDMV and *C. heterostrophus* race O inoculations

For MDMV-A greenhouse studies, seeds were planted in raised soil beds. Beginning at the two- or three-leaf stage, each plant was rub inoculated with MDMV-A on four dates at 2–3 day intervals (Louie 1986). Plants were rated for MDMV-induced symptoms at 2–3-day intervals starting approximately 1 week after the initial inoculation. For MDMV-A field studies, plants were manually inoculated using artist airbrushes powered by a tractor-mounted air pump (Louie et al. 1983). Plants were inoculated four times, starting when most plants in the plots reached the 3rd to 4th leaf stage, with approximately 2 days between inoculations. Plants were rated for MDMV-induced symptoms at 2–3-day intervals beginning approximately 1 week after the initial inoculation.

Inoculum of *C. heterostrophus* race O was produced by growing *C. heterostrophus* race O on lactose-casein agar at 25 °C. After 2 weeks, 10 ml of sterile water was added to each culture plate and a rubber policeman was used to dislodge the conidia. The conidial suspension was then adjusted to approximately 5000 conidia/ml. Plants to be screened for resistance to *C. heterostrophus* race O were planted in raised greenhouse planting beds. At the 3rd to 4th leaf stage, approximately 100 µl (500 conidia) were placed into the leaf whorl using an P-200 Pipetman (Rainin Instrument Inc., Woburn, Mass.). Plants were rated for southern corn leaf-blight symptoms induced by *C. heterostrophus* race O approximately 1 week after inoculation.

RFLP and linkage analysis

All DNA extraction, restriction enzyme digestion, electrophoresis, and autoradiography procedures were as described by McMullen et al. (1994). DNA was transferred to a Genescreen-Plus hybridization membrane (Dupont NEN, Boston, Mass.) using our modification (Simcox and McMullen 1993) of the "dry blot" procedure (Kempter et al. 1991). Table 1 lists the restriction enzymes that detected restriction-site variation among the parents used in this study, and the molecular weights of the polymorphic DNA fragments detected by the RFLP probes. The RFLP probe *bnl6.29A* is a *Sau3*A1-generated

Table 1 Morphological and RFLP alleles of loci used in this study

Locus	Mapping enzyme	Pa405	yM14	*po1* B73	*po1 y1* tester
jc1270	*Eco*RI	9.0[a]	–[b]	7.5	–
npi245	*Hin*dIII	3.5	–	3.0	–
po1		*Po1*	*Po1*	*po1*	*po1*
umc85	*Hin*dIII	8.9	5.0	–	–
	*Eco*RV	6.7	–	2.9	2.9
csu70	*Xba*I	6.3	–	–	3.0
mdm1		*Mdm1*	*mdm1*	*mdm1*	*mdm1*
*p*Zms1	*Eco*RI	9.1, 8.0	9.1	9.1	9.1
	*Sst*I	3.6, 3.2	–	–	3.6, 3.4
bnl6.29A	*Hin*dIII	8.9	8.1	–	–
	*Kpn*I	1.5	–	1.8	1.8
npi235	*Eco*RV	2.6	–	–	5.4
	*Hin*dIII	3.1, 2.5	–	6.4	–
npi101	*Dra*I	3.0	–	11.0, 10.5	–
umc59	*Xba*I	6.0	–	9.0	–

[a] Molecular weight of RFLP allele in kilobase pairs
[b] Probe-enzyme-inbred mapping combination not used in this study

subclone of *bnl6.29* which is specific to a single locus on chromosome 6S (Simcox et al. 1993). The plasmid pZms1 (McMullen et al. 1986), which contains a 3.2-kb *Sau*3A1 fragment of the rDNA spacer region, was used in combination with *Eco*RI or *Sst*I to map the *nor*. In Pa405, pZms1 detects an 8.0-kb fragment, present in approximately 10% of the 26*s* ribosomal DNA (rDNA) genes. The 8.0-kb *Eco*RI restriction fragment was not detected in *y*M14, *po1* B73, or the *po1 y1* tester (Table 1). The rDNA spacer region contains a series of 200-bp subunit repeats and, when digested with *Sst*I, spacer length variation resulting from different numbers of subunit repeats is revealed (Zimmer et al. 1988; Rocheford et al. 1990). The *po1 y1* tester contains a 3.4-kb variant, which is not detected in Pa405, and Pa405 contains a 3.2-kb variant which is not detected in the *po1 y1* tester. Linkage analysis of the BC_1 and F_2 populations was performed and map distances determined using MAPMAKER Version 3.0 for DOS (Lander et al, 1987). Map data scores for the *po1* B73 × Pa405 F_2 population and the recombinant *po1 y1* × Pa405 $F_{2:3}$ families can be obtained through the Maize Genome Database (Coe et al. 1994). Information concerning access to the Maize Genome Database can be obtained from Dennis Hancock (dhancock@teosinte.agron.missouri.edu).

Results

Determination of gene order on chromosome 6S

Previous mapping studies placed *mdm1* proximal to *umc85* and distal to *bnl6.29A* on chromosome 6S (McMullen and Louie 1989), but the location of *mdm1* with respect to the *nor*, a major cytological feature on chromosome 6S, was unknown. Two populations were generated to develop a working map of chromosome 6S: (1) a BC_1 population that took advantage of a recessive white-endosperm factor *y1*, which is linked to the susceptible *mdm1* allele, was used to recover recombinants within this region; and (2) a F_2 population which was used to determine the gene order on chromosome 6S.

A total of 1 488 BC_1 plants were screened in the field for their response to inoculation with MDMV-A. On the basis of segregation of the response to MDMV-A and kernal color, 88 BC_1 plants were identified as having putative crossovers between *mdm1* and *y1*. After greenhouse screening, 53 of the 88 putative recombinants were confirmed to have a crossover between *mdm1* and *y1*. A total of 958 BC_1 progeny, including the 53 confirmed recombinants, were subjected to RFLP analysis. In accordance with our previous report (McMullen and Louie 1989), *mdm1* was located 0.5 cM ($n = 958$) proximal to *umc85* and 0.4 cM ($n = 1488$, excluding possible double crossovers) distal to *bnl6.29A*. The *y1* locus was located 3.3 cM ($n = 1488$, excluding possible double crossovers) proximal to *mdm1*. Using the rDNA spacer probe, pZms1, the *nor* was also mapped in relation to other markers. No crosscovers between *mdm1* and the *nor* were observed in any of the recombinant individuals, indicating that *mdm1* was tightly linked with the *nor*.

To further delineate the order of loci on chromosome 6S, *po1*B73 × Pa405 F_2 progeny were planted and inoculated with MDMV-A in the field. Tissue was collected for RFLP analysis from 187 F_2 individuals which were also scored for susceptibility to MDMV and male sterility (Table 2). Since RFLP analysis was performed directly on the F_2 plants, *mdm1*, *nor*, and *po1* were scored as dominant markers. The order and distance in cM between RFLP and morphological markers on chromosome 6S are as follows; *jc1270*-2.5-*npi245*-1.6-*umc85*/*po1*-0.5-*mdm1*/*nor*-0.5-*bnl6.29A*-0.5-*npi235*-0.8-*npi101A*-4.3-*umc59*. Again, no recombinants were recovered between *mdm1* and the *nor*. Taken together with the BC_1 data, *mdm1* maps either very close to or within the *nor*.

High-resolution map in the region of *Mdm1*

The location of *po1* distal to *mdm1* was used to select for large numbers of recombinants near *mdm1*. Pa405 (*Po1 Mdm1 Y1*) was crossed onto a MDMV-susceptible male-sterile, white-endosperm *po1 y1* tester (*po1 mdm1 y1*) and the resulting F_1 progeny were selfed to generate large numbers of F_2 progeny. Since *po1* and *y1* are only approximately 5 cM apart, homozygous *y1* kernels should yield male-sterile plants, unless a crossover has occurred between *po1* and *y1*. Approximately 7 650 white (*y1*/*y1*) F_2 kernels were planted and 706 fertile individuals (*Po1*-Pa405/–), representing potential recombinants between *po1* and *y1*, were identified and selfed. Ears from 37 out of the 706 fertile $F_{2:3}$ families segregated for *y1* and were discarded. These families may have resulted from heterofertilization events that occur at frequencies of up to 1% (Sarkar and Coe 1971). Of the remaining 669 $F_{2:3}$ families, 626 ears had adequate seed set for further analysis. Out of 7 650 white (*y1*/*y1*) F_2 kernels planted (corresponding to a total of 15 300 chromosomes), 134 $F_{2:3}$ families were recovered which had a crossover between *bnl6.29A* and *po1* (Table 3). The map order and distances in cM, not corrected for double crossovers, of the 134 recombinant F_2:$F_{2:3}$ families is as follows; *umc85*-1.9-*po1*-0.03-*csu70*-0.2-*mdm1*/*nor*-0.8-*bnl6.29A*. The *po1 y1* × Pa405 F_2 map is similar to the results obtained from the BC_1 and F_2 populations, with the exception of *cus70* which was obtained after the BC_1 and F_2 studies were completed. As found

Table 2 F_2 mapping data from the Pa405 × *po1* B73 mapping population

Mapping interval	No. of recombinants	cM[a]
jc1270–npi245	8	2.5
npi245–umc85	6	1.6
umc85–po1	0	0.0
po1–mdm1	1	0.5
mdm1–nor	0	0.0
nor–bnl6.29A	1	0.5
bnl6.29A–npi235	2	0.5
npi235–npi101	3	0.8
npi101–umc59	14	4.3

[a] Map distances determined using MAPMAKER 3.0 for DOS (Landers et al. 1987). Sample population was n = 187 F_2 progeny

Table 3 Pa405 × *poly1* F_2 recombinant screen

Mapping interval	Recombinant F_3s	cM[a]
umc85-pol	5	1.9[b]
pol-csu70	4	0.03
csu70-mdm1	25	0.2
mdm1-Eco RI *nor*	0	0.0
Eco RI-*Sst*I *nor*	0	0.0
nor-bnl6.29A	100	0.7
bnl6.29A-npi235	17	0.1
npi235-y1	483	3.2
pol-y1	638	4.1

[a] Map distances were calculated by determining % recombination out of 15300 chromosomes (7650 F_2 progeny). Values were not corrected for double crossovers

[b] Map distance between *umc85* and *pol* was determined using a total of 268 chromosomes (134 F_2 progeny). Value not corrected for double crossovers

in the BC_1 and F_2 studies, recombination between *mdm1* and the *nor* was not detected. Both of the polymorphic restriction sites used to map the *nor*, the 26*s* rDNA *Eco*RI polymorphism and the *Sst*I spacer length variants, co-segregated with resistance to MDMV. In every case, MDMV-resistant individuals carried the Pa405 8.0-kb *Eco*RI 26 rDNA polymorphism and the Pa405 3.2-kb *Sst*I spacer variant. Concurrently, all MDMV-susceptible individuals were homozygous for the 9.0-kb *Eco*RI 26*s* rDNA polymorphism and the Pa405 3.4-kb *Sst*I spacer variant. No cases of MDMV-susceptible individuals carrying detectable level of the Pa405 *nor* RFLP alleles were identified.

B-A translocation analysis

Whether or not restriction-site variation within the *nor* is arranged in specific domains or as dispersed sites can be addressed using translocations which have breakpoints within the *nor*. The breakpoint of the B-A translocation, TB-6Sa, is located within the *nor* heterochromatin, proximal to a secondary constriction which is associated with the nucleolus (Phillips 1978). Using the recessive *rhm1* locus as a marker, all F_1 progeny resistant to *C. heterostrophus* race O should also be hemizygous for RB37*rhm1* RFLP loci distal to the TB-6Sa breakpoint. As expected, all resistant F_1 progenies were hemizygous for *npi245* and *umc85*, but heterozygous for *bnl6.29A* and *npi235* (Table 4). When probed with pZms1, the 8.0-kb TB-6Sa *Eco*RI 26*s* rDNA polymorphism found in TB-6Sa was not detected in the F_1 hypoploid, indicating that the *Eco*RI 26*s* rDNA polymorphism was located distal to the breakpoint in TB-6Sa. However, when RB37*rhm1* × TB-6Sa F_1 hypoploids were digested with *Sst*I, all of the resistant hypoloid F_1 progeny were heterozygous for the TB-6Sa 3.6- and 3.8-kb subunit repeats and the RB37*rhm1* 3.6-kb subunit repeat (Table 4). This suggests that either all of the *Sst*1 spacer length variants are proximal to the breakpoint in TB-6Sa or else that *Sst*I variation is heterogeneous within the rDNA array.

Discussion

The lack of recombinants between *mdm1* and the *nor* represents a significant problem in attempting a chromosome walk to *mdm1*. Little information concerning the structure of the maize *nor* and the extent of recombination within the rDNA array is available. This lack of of information is due primarily to technical difficulties encountered in attempting to characterize a 14–100 Mb array. The results from the present study suggest three possible relationships between *mdm1* and the *nor*: (1) the resistance we have been attributing to *Mdm1* is a property of the Pa405 *nor*, (2) *mdm1* is located within the *nor*, or (3) *mdm1* is located in the region flanking the *nor* and recombination is suppressed within the region.

There is only one report describing an association between resistance to a plant virus and ribosomal function. Petty et al. (1990) studied the blockage of systemic movement of the Type strain of barley stripe mosaic virus (BSMV) in *Nicotiana benthamiana* by a point substitution in the γ RNA 5′ leader sequence. The point substitution in the Type strain of BSMV creates a short ORF that is not present in the ND18 strain of BSMV which is capable of systemic movement. The authors suggested that the presence of the short ORF reduced translational efficiency of the Type strain, resulting in

Table 4 Morphological and RFLP alleles used to map loci in the TB-6Sa RB37 *rhm1* cross

Locus	Mapping enzyme	TB-6Sa	RB37 *rhm1*	F_1 hypoploids[a]
npi245	*Eco* RI	15.0[b], 14.0, 3.0	6.5	6.5
umc85	*Eco*RI	20.7, 11.6	14.4	14.4
rhm1		*Rhm1*/*Rhm1*	*rhm1*/*rhm1*	*rhm1*/–
pZms1	*Eco* RI	9.1, 8.0	9.1	9.1
	Sst I	3.8, 3.8	3.6	3.8, 3.6
bnl6.29A	*Eco* RI	12.4, 5.0	5.6	12.4, 5.6, 5.0
npi235	*Eco* RI	17.0, 11.6	14.4	17.0, 14.4, 11.6

[a] F_1 hypoploids are hemizygous for all alleles of loci distal to the breakpoint of TB-6Sa on the short arm of chromosome 6

[b] Molecular weight of RFLP allele in kilobase pairs

attenuated infection. In maize, no differences in MDMV-A coat protein titer were observed between resistant and susceptible genotypes at the point of inoculation (authors, unpublished data), suggesting that reduced translational efficiency does not play a role in resistance to MDMV.

The second possibility is that *mdm1* is located within the *nor*. Digestion of the rDNA array using restriction enzymes, such as *Eco*RI, which cut the rDNA array into a single repeat unit suggest that the array is contiguous (McMullen et al. 1986). However, because of the large number of rDNA repeats within the *nor*, the presence of a discrete numbr of non-rDNA sequences may be beyond the limits of detection. While attempting to isolate non-rDNA: rDNA junction fragments from a *Bam*HI partial-size selected λEMBL flax genomic library, Agarwal et al. (1992) recovered a higher-than-expected number of clones containing non-rDNA: rDNA. Based on restriction analysis of these clones, the authors suggested that the flax rDNA array was not contiguous, but may consist of small blocks of rDNA interspersed by single- to low-copy non-rDNA sequences. A similar approach utilizing high-molecular-weight YAC clones (Edwards et al. 1992) from the *nor* might allow determination of whether or not non-rDNA sequences are interspersed within the maize rDNA array.

Another possible explanation for the lack of recombination between *mdm1* and the *nor* is a suppression of recombination caused by the heterochromatic *nor* region. Recombination has been demonstrated to occur within the *Drosophila* rDNA array, but at a much lower frequency than in the surrounding euchromatin (Williams and Robbins 1992). Williams et al. (1989) used flanking morphological and molecular markers to recover recombinantion events within the *Drosophila* rDNA array and determined that recombination occurred primarily within rDNA repeats. There were several instances where recombination within the rDNA array occurred in the absence of flanking marker exchange, suggesting that mechanisms such as gene conversion or double reciprocal exchanges could occur. Williams et al. (1990) also looked at the distribution of spacer variants within the rDNA array and found both clustered and dispersed families of spacer variants. The distribution of the spacer variants suggested that recombination was often unequal and that complex recombination events occurred.

The maize *nor* is composed of a large proximal heterochromatin region, containing an estimated 90% of the rDNA repeats, and a distal secondary constriction containing rDNA associated with the nucleolus (Phillips 1978). The number of rDNA repeats in maize inbred lines varies from 1 650 to 11 500 repeats per haploid nucleus. On the basis of cytogenetic evidence, the transcription of rDNA genes is believed to occur within the secondary constriction (McMullen et al. 1991). The polymorphic *Eco*RI site detected in the 26*s* rDNA of a number of maize inbreds was reported to be associated with hypomethylated and DNase I-hypersensitive sequences (Jupe and Zimmer 1990, 1993). This suggests that the *Eco*RI polymorphism maps to the actively transcribed region of the *nor* in the secondary constriction (McMullen et al. 1991). Localization of the *Eco*RI 26*s* rDNA polymorphism distal to the breakpoint of TB-6Sa lends support to these observations (Table 4).

Although the TB-6Sa data indicated that the 8.0-kb *Eco*RI 26*s* rDNA polymorphisms are clustered distal to the TB-6Sa breakpoint, the *Sst*I data is less informative (Table 4). The TB-6Sa 3.8-kb *Sst*I spacer variant is either clustered proximal to the TB-6Sa breakpoint, or is dispersed within the rDNA array. In the *pol y1* × Pa405 F_2, crossovers within the *nor* should result in a recombinant containing both the Pa405 8.0-kb *Eco*RI 26*s* rDNA allele and the *pol y1* 3.4-kb *Sst*I spacer variant. Since no recombinants of this type were recovered (Table 3) we have no evidence for any recombination within the *nor* in this F_2 population.

The lack of recombination near the *nor* might also have an impact on breeding for resistance to MDMV. Rocheford et al. (1990, 1994) looked at rDNA spacer length variation in populations undergoing selection for grain yield and found that rDNA spacer length variants, and/or associated loci, were influenced by selection. Therefore, resistance to MDMV, and/or linked modifiers, could be indirectly selected along with traits that influence selection within rDNA arrays. A possible demonstration of this indirect selection was shown by Zehr and Wright (1994) who examined genotypic similarities among four inbreds derived at different times from the Iowa Stiff Stalk Synthetic population. These authors found that all four inbreds contained the same alleles at *umc85* and *bnl6.29* even though the inbreds were derived at different times during the development of the population.

Production of a high-resolution genetic map in the region of *mdm1* has not only developed tools for physical mapping of *mdm1*, but has also resolved the map location of *pol* and allowed an examination of recombination within the *nor*. Co-segregation of *mdm1* with the *nor* represents a difficult obstacle in attempting to clone *mdm1* by chromosome walking. Estimates of recombination per kb in maize range from 0.5–1.1 10^{-3} cM/kb over the entire maize genome [based on 3 000 cM (Ed Coe, personal communication) and 2.8–5.7 × 10^6 kb (Galbraith et al. 1983)]. Within-gene estimates of recombination at the *a1* and the *bz1* locus are five-fold higher than the remainder of the genome [*a1* = 4–8 × 10^{-2} cM/kb (Brown and Sundaresan 1991) and 4.6 × 10^{-3}($\pm$ 1.5 × 10^{-3}) cM/kb (Civardi et al. 1994), *bz1* = 7 × 10^{-2} cM/kb (Dooner and Kermicle 1986)] demonstrating the occurrence of recombination "hostspots" within the genome. The *nor* spans an area of 14 to 100 Mb. Assuming *csu70* and *bnl6.29A* flank the *nor*, an estimate of the maximum rate of recombination would be between 0.64–9.0 × 10^{-5} cM/kb. This represents a substantial reduction in the amount of recombination within the 0.9-cM *csu70-bnl6.29A* interval on

the short arm of chromosome six. Our evidence clearly indicates a suppression of recombination at the *nor*. The extent that suppression extends outside the rDNA repeats remains to be determined. This information may be required before we can resolve the position of *mdm1* relative to the *nor*.

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