Forced recombination between distinct strains of *Maize streak virus*

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Recombination between divergent virus genomes is believed to be a major mechanism for generation of novel virus genotypes. We have examined the recombination process in geminiviruses by forcing recombination between two distinct isolates of *Maize streak virus* (MSV), MSV-Kom and MSV-Set. Heterodimeric agroinfectious constructs containing tandemly cloned mixtures of complete or partial MSV-Set and MSV-Kom genomes were used to simulate a circular dimeric form similar to that which would be expected to occur following a single intermolecular crossing-over event between MSV-Set and MSV-Kom replicative form DNAs at the long intergenic region (LIR)–movement protein gene (MP) interface. We isolated, analysed and biologically characterized many of the recombinant MSV genomes that were generated from the constructs *in planta*. Apart from having the same simulated breakpoint at the LIR–MP interface, all the genomes examined had a second breakpoint that had been generated through either intramolecular homologous recombination or a replicational release mechanism. The pathogenicities of six predominantly MSV-Kom-like recombinants were tested in maize. While all were capable of producing a symptomatic infection in this host, none was more virulent than MSV-Kom and only two were more virulent than MSV-Set. The two most virulent recombinants were leafhopper transmitted to a range of differentially MSV-resistant maize, wheat and barley genotypes and both were found to have unique biological properties.

Introduction

*Maize streak virus* (MSV) is the type member of the *Mastrevirus* genus of the single-stranded DNA plant virus family *Geminiviridae*, and is the causal agent of maize streak disease, the most important disease of maize in sub-Saharan Africa (Bosque-Perez, 2000). As with other mastreviruses, MSV has a ~2.7 kb, single component, circular, single-stranded DNA genome containing four open reading frames and two intergenic regions (Fig. 1; Rybicki et al., 2000). Geminiviruses replicate via a rolling circle mechanism (Heyraud et al., 1993a; Saunders et al., 1991; Stenger et al., 1991) with (+)-strand replication always beginning at the penultimate nucleotide of an invariant nonanucleotide sequence (TAATATT $\downarrow$ AC where $\downarrow$ indicates the nick site) within the loop of a conserved hairpin structure [see Gutierrez (1999) for a review of geminivirus replication].

Genomic sequence analyses have revealed substantial evidence for widespread recombination amongst geminiviruses (Berrie et al., 2001; Padidam et al., 1999; Sanz et al., 2000; Zhou et al., 1997, 1998). While it has been reported that recombination is more rare amongst mastreviruses than other geminivirus genera (Padidam et al., 1999), evidence of relatively extensive recombination amongst MSV isolates has recently been presented (Martin et al., 2001). In general, however, recombinant regions detected in mastrevirus genomes have been small relative to those detected for other geminiviruses and, unlike other geminiviruses, mastreviruses do not appear to have a recombination hotspot at their (+)-strand origins of replication (ori).

Whereas the generation of a recombinant linear replicon requires only a single recombination breakpoint, at least two breakpoints are required during the production of recombinant circular replicons. A single intermolecular homologous recombination event between two geminivirus replicative form (RF) DNAs will yield a circular dimeric form (CDF) containing two (+)-strand oris. Release of a recombinant unit length...
Fig. 1. Schematic representation of MSV-Kom/Set homo- and heterodimeric agroinfectious constructs used in this study to force recombination between MSV-Kom and MSV-Set. Unshaded regions represent MSV-Kom sequences and shaded regions represent MSV-Set sequences. Heterodimeric constructs have been named according to the order and amount of MSV-Kom and MSV-Set sequences that they contain: SekA, SekB and SekC constructs contain progressively smaller portions of MSV-Set sequence followed by a full MSV-Kom genome and KosA and KosB constructs contain progressively smaller portions of MSV-Kom sequence followed by a full MSV-Set genome. MP, movement protein gene; CP, coat protein gene; RepA + C2, replication-associated protein gene; LIR, long intergenic region; SIR, short intergenic region. Restriction sites represented here by B, E and Sa refer to BamHI, EcoRI and SacI, respectively.

Both replicational and recombinational release of unit length infectious genomes is believed to occur from geminivirus genome copies tandemly cloned on a Agrobacterium Ti plasmid during agroinoculation (Heyraud et al., 1993a; Stenger et al., 1991). There are a number of reasons why this feature of agroinoculation makes it an ideal tool for analysing geminivirus recombination: (1) the tandemly cloned geminivirus genome copies within an agroinfectious construct are in effect a linear representation of a CDF with the interface between the two genomes in the construct resembling a CDF cross-over site; (2) it is possible to individually simulate all different parts of a CDF using agroinfectious clones by changing the order and amount of viral genomic material that the clones contain; (3) only a very small proportion of the genomes released from heterodimeric agroinfectious clones (agroinfectious clones containing the genomes of two distinct virus genotypes) through recombination will be parental. Chimaeric genomes that are released should therefore face no or very little immediate competition from parental genomes, even when one or both parental genotypes are much fitter than all possible recombinant genotypes.

We have recently described the sequencing and biological characterization of the MSV isolates MSV-Kom and MSV-Set (Schnippenkoetter et al., 2001). MSV-Kom is highly virulent in maize and is an example of the MSV-A strain that is responsible for maize streak disease (Martin et al., 2001). MSV-Set is considerably milder in maize than MSV-Kom and shares only 79% sequence identity with MSV-A isolates. In this paper we describe the use of a series of heterodimeric MSV-Kom/Set agroinfectious constructs to simulate a CDF that would result following an initial crossing-over event between the movement protein gene (MP) and long intergenic region (LIR) of MSV-Kom and MSV-Set. Through sequence analysis of recombinant RF DNAs cloned from agroinoculated plants, we identify the positions of recombination breakpoints and the compositions of mixed recombinant populations. We also analyse the biological properties of a group of the MSV-Kom/Set recombinants and speculate that there is most likely a strong selection pressure to minimize the amount of exogenous sequence within chimaeric genomes.

Methods

Generation of agroinfectious constructs. Full-length agroinfectious MSV-Kom and MSV-Set genomic clones have been described previously (Schnippenkoetter et al., 2001). All DNA manipulations used
standard cloning techniques (Sambrook et al., 1989). A series of homo- and heterodimeric constructs, comprising tandem full- and partial-length MSV-Kom and MSV-Set genomes (Fig. 1), was cloned into the BamHI site of pUC18. EcoRI–XhoI DNA fragments from these clones that contained the homo- and heterodimeric viral genomes were inserted between the corresponding sites of the binary cloning vector pBI121 (Clontech). Heterodimeric constructs were named according to the order of MSV-Kom and MSV-Set sequences that they contained: Whereas SekA, SekB and SekC contained varying amounts of MSV-Set sequence and a full MSV-Kom genome, KosA and KosB contained varying amounts of MSV-Kom sequence and a full MSV-Set genome. Agroinfectious clones of recombinant RF DNAs isolated during this study were constructed in pBI121 as described previously (Schnippenkoetter et al., 2001). All MSV sequences within agroinfectious clones were in the same orientation with respect to vector sequences. Agroinfectious constructs were transformed into Agrobacterium tumefaciens C58C1 (pMP90) (Koncz & Schell, 1986) using the freeze–thaw transformation method of An et al. (1988).

Agroinoculation and symptom quantification. The sweetcorn cultivar Jubilee (Starke Ayres Nursery, Cape Town, South Africa) was used during all agroinoculation experiments. Three-day-old seedlings were agroinoculated and maintained as has been described previously (Martin et al., 1999). The infection rates (IR), degrees of stunting (S) and chlorotic leaf streaking that were elicited by different agroinfectious virus constructs were quantified according to the methods described by Martin et al. (1999).

Detection, cloning and sequencing of recombinant RF-DNAs. MSV RF-DNAs were isolated from symptomatic plants as described by Palmer et al. (1998). The composition of RF-DNA populations was analysed by Southern hybridization using the DIG kit (Boehringer Mannheim). RF-DNAs were digested with RsaI and SacI and restriction fragments were separated by 1% agarose gel electrophoresis before blotting them onto a nitrocellulose membrane. DIG-labelled MSV-Kom and MSV-Set genomes were individually used to probe blots to assess the representation of MSV-Kom and MSV-Set sequences within the RF-DNA populations.

RF-DNAs were randomly cloned into the BamHI site of pUC18. Clones containing MSV genomic DNA were identified by colony hybridization using DIG-labelled MSV-Kom and MSV-Set genomic DNA. Cloned recombinant genomes were isolated and restriction mapped using Apal, BamHI, EcoRI, SalI and XhoI to both establish their identity and to determine the locations of any major recombinant regions.

Clones containing full-length MSV genomes were partially sequenced using an ALF Express automated sequencer (Pharmacia) to determine the positions of recombination breakpoints. Genetics Computer Group software (V7.1; GCG, Wisconsin, USA) was used for all DNA sequence manipulations and analyses.

Leafhopper transmission. Viruses were transmitted by the leafhopper Cicadulina mbila to the maize genotypes sweetcorn, popcorn (Starke Ayres Nursery), Wilplat, Vaalharts Wit (VH Wit), Vaalharts Geel (VH Geel, Summer Grains Centre, Potchefstroom, South Africa), PAN 6549, PAN 6552, PAN 6191, PAN 6363, PAN 6099, PAN 6195 and PAN 6364 (Fannar Seed Co., Greytown, South Africa), the wheat genotypes SST 66, SST 44, Marquis, Dias and Agent, and the barley genotypes Clipper, Adam Tas, Chokka and Festiquay (Elsenberg Agricultural Development Institute, Elsenberg, South Africa). Non-viruliferous leafhoppers were allowed to feed for between 48 and 96 h on agroinoculated maize plants showing streak symptoms. Viruses were transmitted to 2-week-old uninfected maize, wheat and barley seedlings. Viruliferous leafhoppers were allowed to feed on the plants for 5 to 10 days. All transmissions of each virus into particular host genotypes were repeated four times. Disease symptoms were rated on a 5 point rating scale (0 = no streaks and no stunting, 4 = 80–100% of leaf area chlorotic with severe stunting).

Results and Discussion

Agroinoculation of heterodimeric MSV-Kom/Set constructs

We agroinoculated sweetcorn seedlings with a range of fully and partially homo- and heterodimeric MSV-Kom/Set constructs (Fig. 1) and monitored the rates at which plants became symptomatic over a 30 day period. Plants agroinoculated with heterodimers became symptomatic at a slower rate (infection rates from 10–3 for KosA to 37–0 for SekA) than did plants agroinoculated with homodimers (infection rates from 59–3 for MSV-Set to 71–2 for MSV-Kom). Whereas most heterodimer agroinoculated plants only displayed very mild symptoms, certain KosA, SekA and SekB agroinoculated plants developed symptoms on later leaves that resembled either MSV-Set or MSV-Kom.

Rsal and SacI restriction fragment length polymorphisms and Southern blot analysis were used to examine virus populations occurring in symptomatic plants agroinoculated with KosA, SekA and SekB. KosA agroinoculated plants contained predominantly MSV-Set-like RF DNAs and SekA agroinoculated plants contained predominantly MSV-Kom-like RF DNAs (Fig. 2). Whereas minor populations of MSV-Kom and MSV-Set-like RF-DNAs were also detectable in KosA and SekA infected plants, respectively, only MSV-Kom-like RF DNAs were detectable in SekB infected plants (Fig. 2).

Analysis of recombinant RF-DNAs

We cloned, restriction mapped and partially sequenced a total of 36 RF-DNAs from symptomatic plants infected with SekA (8 clones), SekB (11 clones), SekC (5 clones), KosA (9 clones) and KosB (3 clones) to verify the presence of chimaeric MSV-Kom/Set genomes and locate the positions of any recombination breakpoints. Restriction enzyme analysis of the clones and nucleotide sequence analysis of the 500–1700 nucleotides surrounding the MP initiation codon revealed that 33/36 of the clones contained recombinant MSV-Kom/Set genomes. All of the detected recombination breakpoints had occurred within the LIR or MP between 200 nucleotides upstream and 180 nucleotides downstream of the MP start codon. Two of three RF-DNA clones recovered from the KosB infected plant contained wt MSV-Set genomes and one of eight clones recovered from the SekA infected plant contained a wt MSV-Kom genome.

Nine unique chimeric RF-DNAs were identified (Fig. 3): SekA.1, SekA.2, SekA.3, SekA.4 and SekA.5 from a SekA...
infected plant, SekB.1 from a SekB infected plant, and KosA.1, KosA.2 and KosA.3 from a KosA infected plant. Interestingly, clones identical to SekA.1 were also isolated from SekB and SekC infected plants, clones identical to SekA.2 were isolated from a SekB infected plant and a clone identical to KosA.1 was isolated from a KosB infected plant.

All but one of the recombination breakpoints that were not at the (+)-strand ori were in regions of the genome where MSV-Kom and MSV-Set share complete nucleotide sequence identity over stretches of nine or more nucleotides (Fig. 4).

Four out of five of these regions were implicated in two or more independent recombination events (Fig. 4).

Recombinant regions ranged in size from 2 to 178 nucleotides and in all cases the boundary between MSV-Kom and MSV-Set genomes within the heterodimeric constructs was one of the recombination breakpoints (hereafter referred to as the forced recombination breakpoint). All the SekA, SekB and SekC derived recombinants contained predominantly MSV-Kom sequence with between 2 and 170 nucleotides of MSV-Set sequence in their LIRs upstream of the forced
Forced recombination between MSV strains

<table>
<thead>
<tr>
<th>Agroinfectious construct</th>
<th>Position in genome</th>
<th>Percentage of clones</th>
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<tbody>
<tr>
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<td></td>
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<tr>
<td>SekA.1</td>
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<td></td>
</tr>
<tr>
<td>SekA.2</td>
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<td></td>
</tr>
<tr>
<td>SekA.3</td>
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<tr>
<td>SekB.1</td>
<td>147</td>
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<tr>
<td>SekA.1</td>
<td>44</td>
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<td>SekB.2</td>
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<td>SekC.1</td>
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<tr>
<td>MSV-Set</td>
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Fig. 3. A schematic representation of the LIR (box) and MP (arrow) regions of recombinant MSV-genomes and the proportions in which these genomes were isolated from heterodimer agroinoculated plants. MSV-Kom-derived sequences are in white and MSV-Set-derived sequences are in black. The origin of (+)-strand replication and the forced recombination breakpoint are at nucleotide sequence positions 1 and 149, respectively. The positions of recombination breakpoints are indicated and nucleotide sequence insertions are represented by '. Note that although recombinants are generally named after the heterodimeric agroinfectious construct from which they arose, clones identical to SekA.1, SekA.2 and KosA.1 were isolated from plants agroinoculated with two or more different heterodimers.

recombination breakpoint. Whereas KosA derived recombinants containing predominantly MSV-Kom or MSV-Set sequences were recovered, the only KosB derived recombinants that were recovered contained predominantly MSV-Set sequences. The predominantly MSV-Kom chimaeras, KosA.2 and KosA.3 respectively, contained 25 and 178 nucleotides of MSV-Set sequence in their MP genes directly downstream of the forced recombination breakpoint. The predominantly MSV-Set chimaera KosA.1 that was recovered from both KosA and KosB infected plants contained 2 nucleotides of MSV-Kom sequence upstream of the forced recombination breakpoint.

Assuming that homologous recombination between MSV-Kom and MSV-Set genomes requires that they share at least two identical nucleotides at the cross-over site, there are a total of 334 sites at which these genomes can recombine. Whereas all 334 sites were available for recombinational release of unit length genomes from the SekA and KosA heterodimers, 114 sites could be used in the SekB heterodimer and 31 in the SekC and KosB heterodimers. Only six of these sites, all of which were within 200 nucleotides of the forced recombination breakpoint, were involved in the recombinational generation of all the genomes that we isolated from heterodimer inoculated plants (Fig. 4).

While it might appear that this genomic region surrounding the forced recombination breakpoint constitutes a recombination ‘hot spot’, it is also possible that selection in planta has favoured the survival of recombinants that most closely resemble wt MSV-Kom or MSV-Set genomes. The appearance of apparent recombination hot spots under conditions of selection that are otherwise absent when selection is removed has been observed in recombination studies of the coronavirus Mouse hepatitis virus (Banner & Lai, 1991). Our results indicate that selection has influenced the pattern of recombination that we observed. MSV-Kom is more pathogenic in maize than MSV-Set (Schnippenkoetter et al., 2001). Accordingly, whenever agroinfectious heterodimers contained a full MSV-Kom genome (SekA, SekB, SekC and KosA), predominantly MSV-Kom-like genomes dominated the populations of clones that were recovered from agroinoculated plants. It is conceivable therefore that recombinants with more equitable portions of MSV-Kom and MSV-Set sequence may have been produced in heterodimer inoculated plants but that they were not detected because they were outcompeted by the more viable mostly MSV-Kom or MSV-Set-like recombinants that we did detect in these plants.

Only three of the nine unique chimaeras that we identified (SekA.3, SekA.4 and SekA.5) had recombination breakpoints in close proximity to the (+)-strand ori and were, therefore, potentially the direct products of replicational release from heterodimeric agroinfectious constructs (SekA in this case). All of the other chimaeras had apparently arisen through either direct recombinational release from heterodimeric agroinfectious constructs or homologous recombination between genomes that had already been replicationally released from these constructs.

It is, however, uncertain whether SekA.3, SekA.4 and SekA.5 could have originated through the conventionally accepted replicational release mechanism proposed by Stenger et al. (1991). Whereas replicational release according to this model would be expected to yield chimaeras with recombination breakpoints precisely at the (+)-strand ori, (see the
Fig. 4. Homologous recombination breakpoints (boxed sequences) that were detected within the LIR and MP sequences of the various MSV-Kom/Set chimaeras identified in this study. Names of chimaeras or wt genomes released through homologous recombination within the boxed regions are presented above the boxed regions. When considering the various chimaeras observed and the different plants from which they were recovered the event numbers above boxed regions refer to the minimum number of independent homologous recombination events that had to have occurred within these regions. The forced recombination breakpoint at the boundary between the MP and LIR is indicated in bold. The TAATATTAC nonanucleotide at the (-)-strand origin of replication is underlined.

Fig. 5. Alignment of nucleotide sequences at the (-)-strand origins of replication (ori) of MSV-Kom, MSV-Set and chimaeric MSV genomes that were potentially the products of replicational release from the heterodimeric agroinfectious construct, SekA.3. Also presented here is a theoretical chimaeric sequence that should have been replicationally released from SekA according to the replicational release model of Stenger et al. (1991). Sequences of MSV-Set origin are in bold. The TAATATTAC nonanucleotides characterizing all geminivirus (-)-strand oris are presented in italics. The reverse complementary left and right ‘stem’ sequences facilitating the formation of a stable hairpin structure at the (-)-strand ori are indicated. Identical MSV-Kom and MSV-Set stem sequences are boxed. Paired nucleotide sequence mismatches that would destabilize a hybrid MSV-Kom/Set hairpin structure are numbered 1 through 4. The TTACC nucleotide sequences which are potentially involved in the replicational release process from a hybrid MSV-Kom/Set stem–loop sequence are underlined.

‘theoretical’ sequence in Fig. 5), the breakpoints in SekA.3, SekA.4 and SekA.5 were between 11 and 15 nucleotides downstream of the ori. In all three clones, the breakpoint region included the nucleotide sequence TTACC – a sequence which is identical to that surrounding the (-)-strand ori. Interestingly, SekA.4 and SekA.5 respectively contain 13 and 11 nucleotide sequence insertions at their recombination breakpoints. In both cases the insertions are bounded by TTACC residues (Fig. 5).

The nucleotide insertions in SekA.4 and SekA.5 were probably the products of erroneous termination of (-)-strand replication. Initiation of replicational release from the SekA heterodimer would have been from the (+)-strand ori within the MSV-Set LIR (Fig. 1). There are two reasons to believe that the MSV-Set Rep would have initiated this process: (1) neither the SekB nor SekC heterodimers contained a full MSV-Set Rep and no replicationally released recombinants were detected in plants infected with these heterodimers; (2) while MSV-Kom is capable of trans-replicating MSV-Set, the MSV-Kom Rep interacts very poorly with the MSV-Set LIR (Willment, 1999). For replicative release of SekA.4 and SekA.5 from the SekA heterodimer to have proceeded past the MSV-Kom (+)-strand ori, an alternative site 11–13 nucleotides downstream of the
MSV-Kom ori must have been nicked by either an MSV-Kom or MSV-Set Rep molecule. Alternative Rep nicking sites are not unprecedented amongst the mastreviruses. Rep of Wheat dwarf virus (WDV) has the capacity to initiate (+)-strand replication at two separate points within the WDV LIR – one at the conventional (+)-strand ori and the other ~170 nucleotides upstream from the ori (Heyraud et al., 1993b). The alternative nicking sites within the SekA heterodimer would have been immediately upstream of a region where the 5’ MSV-Kom stem sequence has a high degree of complementarity with the 3’ MSV-Set stem sequence.

SekA.3, the recombinant with a breakpoint near the ori but without any nucleotide insertions, is either the direct product of recombinational release from the SekA heterodimer or the modified progeny of a replicationally released genome that contained a hairpin stem insertion. Whereas there is evidence that during the generation of SekA.4 and SekA.5 replication had been initiated from the expected position in the MSV-Set TAATATTAC (+)-strand ori sequence, if SekA.3 was the direct product of replicational release from SekA it would imply that replication had to have been initiated from the TTAC sequence within the 3’ stem of the MSV-Set ori hairpin. It is, however, possible that SekA.3 is the deletion product of a recombinant resembling SekA.4 or SekA.5. Six and nine nucleotide direct repeats surrounding the nucleotide insertions in SekA.4 and SekA.5, respectively (Fig. 5) may have facilitated homologous recombinational deletion of these insertions. In the case of SekA.4, homologous recombination between the directly repeated sequences would result in the generation of SekA.3.

**Biological characterization of recombinant genomes**

To test the viability of the predominantly MSV-Kom-like recombinants which contained over 100 nucleotides of MSV-Set sequence, we constructed agroinfectious clones of SekA.1, SekA.2, SekA.3, SekA.4, SekA.5 and KosA.3 and examined the symptoms that they produced in agroinoculated sweetcorn plants (Fig. 6).

The most pathogenic of the chimaeras tested were KosA.3 and SekA.1. Both produced greater chlorotic areas and induced more stunting in sweetcorn than did MSV-Set. While they had similar infection rates to MSV-Set and MSV-Kom, they induced less stunting and produced smaller chlorotic areas than did MSV-Kom (Fig. 6). SekA.2 was approximately as virulent as MSV-Set but had a slightly lower infection rate. SekA.3, SekA.4 and SekA.5 all had lower infection rates and produced smaller chlorotic areas in symptomatic plants than did MSV-Set.

It is probable that differences in pathogenicity between SekA.1, SekA.2, SekA.3, SekA.4 and SekA.5 are due entirely to differences in the sequences that they contain in the immediate vicinity of the (+)-strand ori. SekA.1 contains an ori hairpin sequence identical to that of MSV-Kom, SekA.2 contains a hairpin sequence identical to that of MSV-Set, and SekA.3, SekA.4 and SekA.5 contain hybrid MSV-Kom/Set hairpin sequences. It has been demonstrated using WDV that Rep oligomers bind with low affinity to the (+)-strand ori hairpin (Castellano et al., 1999). It is therefore feasible that the SekA.2, SekA.3, SekA.4 and SekA.5 are less pathogenic than SekA.1 because MSV-Kom Rep molecules (which all of these recombinants express) interact suboptimally with MSV-Set and hybrid MSV-Kom/Set ori sequences.

In addition to having potentially altered MSV-Kom Rep binding sequences, the SekA.3, SekA.4 and SekA.5 hybrid ori sequences would produce altered hairpin structures that are substantially less stable than those predicted for MSV-Kom and MSV-Set. It is perhaps not surprising therefore that these recombinants were less pathogenic than SekA.1 and SekA.2. It is, however, interesting that both SekA.4 and SekA.5 have nucleotide insertions within their hybrid ori sequences and that both are significantly more pathogenic than SekA.3, which contains no nucleotide insertions. While the most stable secondary structures predicted for the SekA.4 and SekA.5 ori sequences are more stable than that predicted for the SekA.3 sequence (data not shown), further experiments will be required to establish exactly how these small differences in sequence translate into such large differences in pathogenicity.

Our results may indicate why no naturally occurring mastrevirus recombinants with breakpoints within the (+)-strand ori hairpins have been discovered. Mastrevirus hairpin stem sequences are far less conserved than those of begomoviruses and curtoviruses. If mastreviruses sharing less than ~85% nucleotide sequence identity recombine, our sequence data analysis suggests that genomes that are replicationally released from CDFs will tend to have nucleotide mismatches within the stem sequences of their (+)-strand ori hairpins. In a study involving the begomovirus Tomato golden mosaic virus, mutations that introduced nucleotide mismatches into the stem sequences of (+)-strand ori hairpins adversely affected (+)-strand replication (Orozco & Hanley-Bowdoin, 1996). Although we have shown here that recombinants with hybrid hairpin stems are viable, we have also demonstrated that they are severely defective relative to their parental viruses and would most likely never survive for long enough in nature to be detected.

We investigated the impact of recombination on the biological characteristics of SekA.1 and KosA.3 in greater depth by leafhopper transmitting these viruses from agroinoculated highly symptomatic sweetcorn plants to a range of barley, wheat and differentially MSV-resistant maize genotypes. Because both chimaeras contained predominantly MSV-Kom sequence we were interested in determining whether the small portions of MSV-Set LIR (SekA.1) or MP (KosA.3) sequence they contained had altered their fitness relative to MSV-Kom in these hosts. The chimaeras did not induce any symptoms in the barley or wheat genotypes that were immune to symptomatic infection by MSV-Kom (Festi quy, Agent and
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**Fig. 6.** Symptoms observed in sweetcorn plants agroinoculated with MSV-Kom, MSV-Set and various recombinant viruses isolated or produced during this study. (A) The mean percentage chlorotic area observed on leaves one through six of symptomatic plants infected with MSV-Kom (●), MSV-Set (○), KosA.3 (▼), SekA.1 (▲), SekA.2 (■), SekA.3 (●), SekA.4 (△) and SekA.5 (◆). (B) The number of plants showing symptoms between days 5 and 14 after agroinfection, assessed at four time-points, was averaged to give the infection rate (IR) for each isolate. (C) Stunting of symptomatic plants expressed as $1 - \text{(height of symptomatic plants)/(height of uninfected control plants)}$. Error bars represent 95% confidence intervals of means (using Student’s t-test).

**Fig. 7.** Relative severities of symptoms produced by MSV-Kom, KosA.3, SekA.1 and MSV-Set in a range of differentially MSV sensitive barley, wheat and maize genotypes. A symptom rating of 0 implies plants were asymptomatic and a rating of 4 implies > 80% chlorosis and severe stunting.

SST-44; Fig. 7). Both of the chimaeras did, however, produce streak symptoms that were noticeably more severe than those produced by MSV-Kom in the barley genotype Chokka and the wheat genotype Dias (Fig. 7).

Both chimaeras generally produced milder symptoms than did MSV-Kom in maize, except that in Witplat KosA.3 produced symptoms that were indistinguishable in severity from those produced by MSV-Kom. Interestingly, whereas KosA.3 produced more severe symptoms than did SekA.1 in maize genotypes in which MSV-Set induced symptoms with a severity rating of two or more (Sweetcorn, popcorn and Witplat), SekA.1 produced symptoms indistinguishable from or noticeably more severe than those produced by KosA.3 in most maize genotypes in which MSV-Set induced symptoms with a severity rating of less than one (PAN6549, PAN6364, PAN6542, PAN6099, PAN6191 and VH Geel; Fig. 7). Because KosA.3 contains MSV-Set sequences in the 5’ ~ 40% of its MP gene, this result would indicate that sequences in this
genomic region are at least partially responsible for the inability of MSV-Set to symptomatically infect most of the MSV-resistant maize genotypes examined here. While MSV-Set may simply have a poorly maize-adapted MP gene, it is also possible that one of the many uncharacterized resistance genes present within certain MSV-resistant maize genotypes interferes with MP function.

It has been speculated that recombination between geminiviruses has been a major contributing factor behind the recent emergence of a number of devastating crop diseases worldwide (Padidam et al., 1999). The long-term survival and establishment of a recombinant virus genotype in nature will depend strongly on it having a selective advantage over the overwhelming populations of its parental genotypes. We have shown here that a small subset of all possible chimaeras that could result following an initial crossing-over event between the MP gene and LIR of two different MSV strains is viable with biological characteristics that are distinct from those of the parental strains. Although none of the MSV-Kom/Set recombinants that we have dealt with here was more pathogenic than both MSV-Kom and MSV-Set in any of the host species and genotypes we examined in our results help explain the patterns of natural mastrevirus recombination noted previously. Our failure to detect recombinants containing more than ~200 nucleotides of exogenous sequence fits well with sequence evidence that interstrain MSV recombinants generally contain fewer than ~150 nucleotides of exogenous sequence (Martin et al., 2001). The results presented here on the relative pathogenicities of recombinants with breakpoints at and around the (+)-strand ori provide an explanation for why this region is less of a recombination hot spot in mastreviruses than it apparently is in begomoviruses. We are currently using many of the MSV-Kom/Set recombinants described here together with analogous laboratory-constructed chimaeras to determine the positions and affinities of the replication specificity determinants within the LIRs of different MSV strains.

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References


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