Development of Infectious Clones of Sugarcane Mosaic Virus

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INTRODUCTION

Plant viruses cause significant disease on crops and can result in devastating economic losses. Of the more than 50 viruses identified in maize, at least 12 cause severe agronomic problems globally[1]. Among these, sugarcane mosaic virus (SCMV) is perhaps the most prevalent and widely distributed worldwide. SCMV is a +ssRNA picorna-like virus in the family *Potyviridae*, genus *Potyvirus*, which encodes a large polyprotein cleaved by three viral proteases into ten functional viral proteins (Fig.1)[2]. The single genomic RNA is encapsidated by the coat protein to form flexuous rod virions, which are transmitted non-persistently by aphids. SCMV systemically infects plants and causes stunting and mosaic symptoms, and reduction in crop yield for important agricultural crops including maize, sugarcane, and sorghum[3]. SCMV often co-infects with other viruses in disease complexes including the devastating emergent synergistic disease maize lethal necrosis (MLN)[4&5].

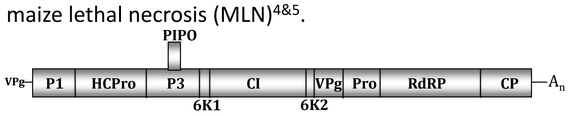

Figure 1. Genome organization of SCMV. Three proteinases (P1, HC-Pro and Pro) cleave the polyprotein into 10 mature functional proteins.

Two SCMV isolates, one from Ohio (SCMV-OH) and one from Germany (SCMV-GR) were selected to develop infectious clones because of their differential response to maize resistance genes. Unlike SCMV-OH, SCMV-GR is not able to infect NIL *Wsm1* carrying a single potyvirus R-gene[6]. SCMV-GR shares 79% nucleotide and 89% polyprotein amino acid sequence identity with SCMV-OH, which fall into separate groups of SCMV isolates (Fig. 2). Sequences are most polymorphic within P1 and CP N-terminal coding regions (Fig. 3). We report here development of full-length infectious clones of both isolates of SCMV-OH and SCMV-GR, which will allow future mapping of viral resistance response determinants.

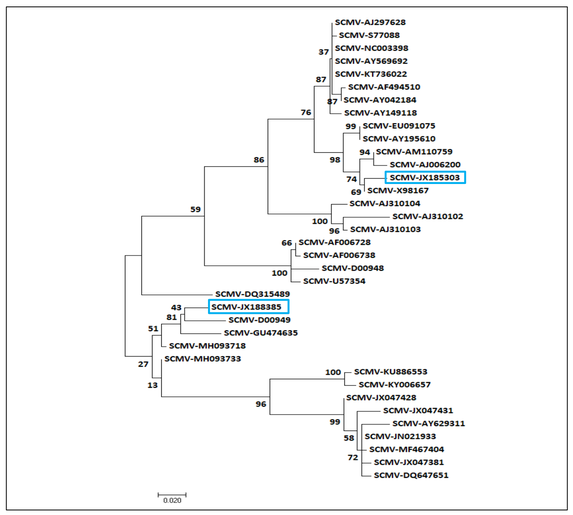

Figure 2. Phylogenetic tree of amino acid sequences of coat protein of SCMV isolates. SCMV-OH (JX188385) and SCMV-GR (JX185303).

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SCMV-OH   1   SGTVDAGTQGGSGSQGTTPPATGSGAKPAT--SGAGS-GSSTG-AGTGVTGSQAGAGGSA  56
SCMV-GR   1   SGSVDAGAQGGNSGSGASTPAAGSGSGTRPPSTGSAAQGN-TPTASGGSSGNNGSNQ-SG  58

SCMV-OH  57   GTGSGA--TGGQSGSGSGTGQINTGSAGTSATGGQRDRDVDAGTTGKISVPKLKAMSKKM  114
SCMV-GR  59   SNGTGNQAGASGTGDQRDKDVDV-GS------------------TGKISVPKLKAMSKKM  99

SCMV-OH  15   RLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEEFDRWYDAIKKEYEIDDTQMTVVMSG  174
SCMV-GR  100  RLPKAKGKDVLHLDFLLTYKPQQQDISNTRATKEEFDRWYDAIKKEYEIDDTQMTVVMSG  159

SCMV-OH   75   LMVWCIENGCSPNINGNWTMMDGDEQRVFPLKPVIENASPTFRQIMHHFSDAAEAYIEYR  234
SCMV-GR  160   LMVWCIENGCSPNINGNWTMMDGNEQRVFPLKPVIENASPTFRQIMHHFSDAAEAYIEYR  219

SCMV-OH  235   NSTERYMPRYGLQRNLTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFG  294
SCMV-GR  220   NSTERYMPRYGLQRNLTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFG  279

SCMV-OH  295   LDGNVGETQENTERHTAGDVSRNMHSLLGVQQHH  328
SCMV-GR  280   LDGNVGETQENTERHTAGDVSRNMHSLLGVQQHH  313
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Figure 3. Comparison of 5' terminal region of amino acid sequences of coat proteins of SCMV-OH and SCMV-GR.

MATERIALS & METHODS

Virus samples were originally collected in Ohio (ca. 1970) and Seehausen, Germany and maintained by rub-inoculation. Total RNA was isolated and complementary DNA (cDNA) was synthesized using oligo-dT primers. Terminal sequences of SCMV-OH and SCMV-GR were determined using 5'- & 3'-RACE. SCMV full-length cDNA was amplified using WX263 and WX264 primers, cloned into a binary vector pJL89[7] and partially sequenced. SCMV full-length DNA template was prepared by PCR using WX241 and WX245 primers for RNA transcript synthesis using the HiScribe™ T7 ARCA mRNA Kit (New England BioLabs), and subsequently for vascular puncture inoculation (VPI) into maize seeds[8] (Fig. 4). SCMV infection was confirmed by symptoms scoring and reverse transcription-polymerase chain reaction (RT-PCR) with SCMV-specific primers.

Figure 4. VPI delivery of SCMV transcripts into maize seeds.

RESULTS

RACE analysis indicate an additional nine nucleotides (5'-GTGAGAGAC-3') are present at 3' most terminal region from the published nucleotide sequences of both SCMV-OH (JX188385) and SCMV-GR (JX185303). The 5' most nucleotide of SCMV-OH is adenine instead of uracil, consistent with other published SCMV isolates. Twenty-five additional nucleotides (5'-AAAAACAACAAAACTC-AACACAACA-3') are present which are absent at 5' terminal region of the reported SCMV-GR isolate sequence. Thus, the updated sequences of SCMV-OH and SCMV-GR are 9622 and 9583 nucleotides respectively.

Two full-length SCMV-GR cDNA clones, pWX53-54, were obtained (Fig. 5). Clone pWX54 was verified by sequencing of 5' terminal region of SCMV-GR (1-886) and 3' terminal region (8743-9583). VPI of *Z. mays* cv. Silver Queen using RNA transcripts derived from clone pWX54 resulted in typical mosaic symptoms (Fig. 6). SCMV infection was confirmed by RT-PCR analysis of symptomatic plants using SCMV-specific primers to yield expected amplified DNA of 596 bp (Fig. 7). SCMV-GR infection was further verified by sequencing the PCR amplified DNA at both 5' terminal region of SCMV (1-1159) and 3' terminal region (8129-9536) from symptomatic infected plants.

Four full-length cDNA clones of SCMV-OH in pJL89 were also obtained (pWX58 to pWX61). Clones were verified by sequencing of 5' terminal region of SCMV-OH (1-1159) and 3' terminal region (8530-9622). Typical mosaic symptoms were observed from VPI of *Z. mays* cv. Silver Queen using RNA transcripts derived from clones pWX58 and pWX60. RT-PCR analysis of symptomatic plants confirmed SCMV-OH infection (Fig. 8).

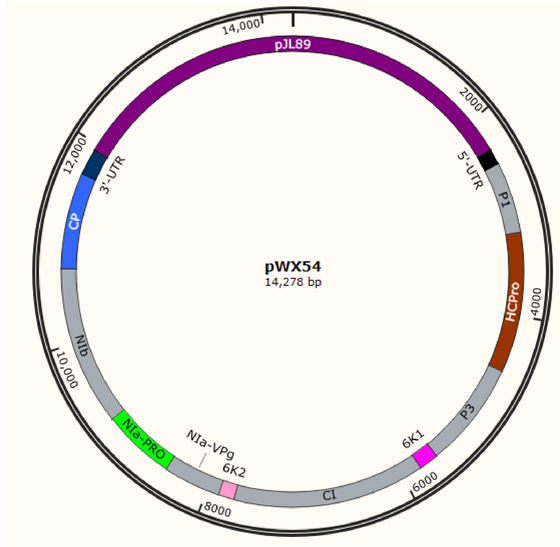

Figure 5. Map of SCMV-GR in pJL89 (pWX54).

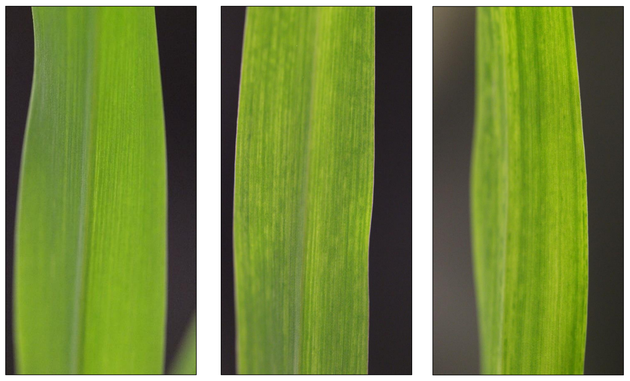

Figure 6. Symptom of SCMV-GR (pWX54) and wild type SCMV-OH infection versus healthy plant leaves.

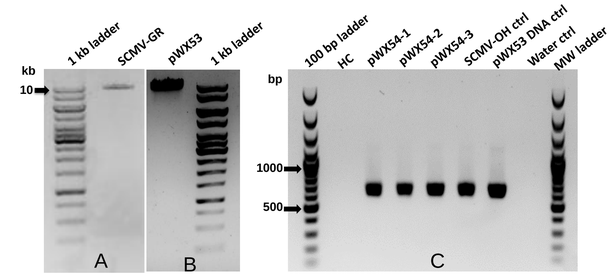

Figure 7. Full-length cDNA amplification of SCMV-GR by PCR for cloning into pJL89 (A), full-length viral DNA amplification of pWX53 for *in vitro* transcript synthesis (B), and RT-PCR analysis of VPI infected maize plants (C). SCMV-specific primers were used for SCMV infection assay. HC: healthy plant; MW ladder: 100 bp ladder.

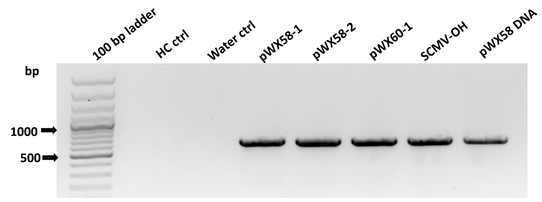

Figure 8. RT-PCR analysis of VPI infected maize plants. HC: healthy plant control; SCMV-OH: SCMV-OH wild type infected plant control; pWX58-1&2: pWX58 derived infected plants; pWX60-1: pWX60 derived infected plant; pWX58 DNA: plasmid DNA ctrl; 596 bp DNA amplified with SCMV-specific primers of WX237/WX238.

CONCLUSIONS & DISCUSSION

Infectious full-length cDNA clones were obtained for both SCMV Ohio isolate (SCMV-OH) and German isolate (SCMV-GR), and they can be used as backbone vectors for gene expression or virus-induced gene silencing (VIGS) constructs. Development of the infectious clones allows us to next determine the molecular basis of differential host range or resistance-breaking of SCMV-OH. A previous report demonstrated SCMV-GR was not able to infect some maize breeding lines susceptible to SCMV-OH. As SCMV-OH and SCMV-GR genome sequences differ primarily in P1 and coat protein, the use of mutational analysis and/or gene swap strategy between these two SCMV isolate genomes is currently under investigation to pinpoint any viral gene pathogenic determinants, and the resistance-breaking mechanism of SCMV-OH.

ACKNOWLEDGEMENTS

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