Cross-Protection Studies Between Strains of Sugarcane Mosaic, Maize Dwarf Mosaic, Johnsongrass Mosaic, and Sorghum Mosaic Potyviruses

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ABSTRACT

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Cross-protection was studied between strains of viruses comprising the sugarcane mosaic virus (SCMV) subgroup, namely Johnsongrass mosaic, maize dwarf mosaic, sorghum mosaic, and SCMV, in 53 different combinations using differential hosts and Western blot immunoassays. Cross-protection occurred only between SCMV-MDB and SCMV-BC when the former was inoculated first and the latter used as the challenge strain, but neither vice versa nor in any other combinations including the 19 that involved recognized strains of the one virus, SCMV. The unidirectional protection between SCMV-MDB and SCMV-BC and the negative cross-protection results between other strains of SCMV appear to correlate with different sequence motifs present in the hypervariable region of the coat protein N-terminus of the SCMV strains.

Sugarcane mosaic (SCMV), maize dwarf mosaic (MDMV), Johnsongrass mosaic (JGMV), and sorghum mosaic (SrMV) viruses, formerly considered strains of SCMV, are now classified as distinct viruses (13,25,30). The new taxonomy of these potyviruses was based on amino acid sequences (21,23), amino-terminal serology (24,25), cell-free translation of RNA (3), reactions of differential sorghum and oat cultivars (9,30), peptide profiling of coat proteins (13), molecular hybridization with probes corresponding to the 3′ noncoding regions (5), and the morphology and serology of cytoplasmic cylindrical inclusions (7,9,11).

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It is well-known with plant viruses in general that mixed infections may result in cross-protection. Since a positive cross-protection result generally indicates a strain relationship (12,26), the identities and the relationships among different viruses and strains can be evaluated by cross-protection (6,12,25). Previous cross-protection studies between the former SCMV strains resulted in either complete protection (16), partial protection (1,19,28,31), or no protection (8,17). Due to the extreme diversity of the previous cross-protection results and the fact that the former SCMV strains now comprise four distinct potyviruses (21,25), it was of interest to re-examine the cross-protection effects between potyviruses and their strains which infect maize, sugarcane, and sorghum around the world. Here, we present results obtained from an investigation of cross-protection between strains of JGMV, MDMV, SCMV, and SrMV.

MATERIALS AND METHODS

The viruses and strains between which cross-protection was studied were as follows: SCMV-A, -B, -D, and -MDB (isolate IA 66-188) from the United States, and -BC, -SC, and -Sabi from Australia; MDMV-A (isolate IA 65-74) from the United States; JGMV-JG from Australia; and SrMV-SCH and -SCI (former SCMV-H and -I) from the United States. All virus isolates were the same as those investigated by Tosic et al (30). In addition, both MDMV-Yu, an isolate from Yugoslavia identical to MDMV-A (9,30), and SCMV-Yu, which was isolated recently from maize in

Yugoslavia and identified as a new strain of SCMV (9), were also included in this study.

The strains of viruses were maintained and propagated in sweet corn cv. Gold Cup. Seedlings of Gold Cup were used also as test plants to evaluate cross-protection. Cross-protection was studied in sequential first and second (challenge) inoculations, after which the second inoculated virus was assayed for its multiplication in inoculated seedlings. The first virus or strain was inoculated mechanically onto the second leaf of sweet corn seedlings in the three-leaf stage. The second virus or strain was then inoculated mechanically 10 days later onto the second leaf from the top. This challenge virus was inoculated only onto the seedlings showing symptoms caused by the first inoculated virus. The challenge inoculation was done with a virus or strain that could be differentiated from the first inoculated virus by reaction of specific sorghum and/or oat cultivars (9,30) or by serology (25,29). The assay for the challenge-inoculated virus was done 15 days after the challenge inoculation using tissue from the youngest leaf of the test seedlings. Cross-protection was studied between the virus strain combinations listed in Table 1.

Multiplication of the challenge virus was checked by mechanical inoculation for bioassay as well as by serological reaction. The bioassay plants used as indicators of the challenge virus are listed in Table 2. These sorghum and oat cultivars are ones previously found to differentiate strains of viruses in the SCMV subgroup (30). In addition, the sorghum cv. IS 8642, now recommended (9) for the differentiation of SCMV-MDB from SCMV-D, was also used. Mechanical inoculation was the method of choice.

Serological analysis of the challenge virus was performed using antisera to the following viral strains: MDMV-A prepared against the isolate IA 65-74 (29) and cross-absorbed with SCMV-MDB; SCMV-MDB prepared against the isolate IA 66-188 (29) and cross-absorbed with SCMV-Yu; SCMV-D (29) cross-absorbed with SCMV-Sabi; SCMV-Yu prepared against the SM-3 isolate from Yugoslavia, temporarily designated the Yu strain (9) and cross-absorbed with SCMV-MDB; and JGMV-JG (24). The JGMV-JG antiserum reacted only with the homologous antigen (24). Therefore, cross-absorption was not necessary for this antiserum. Cross-absorption of antisera was performed as follows (29): to 0.3 ml of antiserum, 0.1 ml of infective plant sap was added, and the mixture was incubated for 1 hr at 38–40 C, then centrifuged for 30 min at 3,000 *g*. The cross-absorption was repeated until no further reaction occurred with the antigen used for cross-absorption.

Serological analysis was done by Western blot immunoassay, based on previously described procedures (15,18, 24). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed (10) with the electrophoresis apparatus from Hoefer Scientific Instruments Co. (San Francisco, CA), model SE 400. Forty-microliter samples prepared from infective plant sap were applied to each slot of the gel. Electrophoresis at room temperature was run first at a setting of 20 mA and 200 V during protein concentration, then at 40 mA and 500 V to finish the run. After electrophoresis, the protein bands were blotted onto nitrocellulose strips of 0.45 μm, and the blotted proteins were subjected to an immunoenzyme treatment as described by Shukla et al (24).

Table 1. Combination of strains of JGMV, MDMV, SCMV, and SrMV used in cross-protection studies

Challenge strain	First inoculated strain
Combinations involving strains of distinct viruses	
JGMV-JG	MDMV-A or -A (Yu)
JGMV-JG	SCMV-A, -D, -SC, -Sabi, -BC, -MDB, or -Yu
JGMV-JG	SrMV-SCH or -SCI
MDMV-A	JGMV-JG
MDMV-A	SCMV-A, -B, -D, -SC, -Sabi, -BC, or -Yu
MDMV-A	SrMV-SCH or -SCI
MDMV-A (Yu)	SCMV-MDB or -Yu
SCMV-D	JGMV-JG
SCMV-MDB	JGMV-JG
SCMV-MDB	MDMV-A or -A (Yu)
SCMV-MDB	SrMV-SCH or -SCI
SCMV-Yu	JGMV-JG
SCMV-Yu	MDMV-A or -A (Yu)
SCMV-Yu	SrMV-SCH or -SCI
Combinations involving strains of one virus	
SCMV-BC	SCMV-MDB
SCMV-D	SCMV-SC, -BC, or -Sabi
SCMV-MDB	SCMV-A, -D, -SC, -Sabi, -BC, or -Yu
SCMV-Sabi	SCMV-MDB
SCMV-SC	SCMV-MDB
SCMV-Yu	SCMV-A, -B, -D, -SC, -Sabi, -BC, or -MDB

Table 2. Sorghum and oat cultivars used for bioassay of the challenge viral strain

Challenge strains	Cultivars used
SCMV-MDB	Sorghum cvs. Atlas, NM 31, Tamaran, and IS 8642
MDMV-A, -A (Yu)	Sorghum cvs. Rio, R 430, Atlas, and TX 2786
JGMV-JG	Oat cv. Garland; sorghum cvs. Trudex, TX 2786, NM 31, and Atlas
SCMV-Yu	Sorghum cvs. Atlas, NM 31, Trudex, Tamaran, and OKY 8
SCMV-D	Sorghum cvs. R 430, Atlas, and BTX 398
SCMV-SC, -BC, -Sabi	Sorghum cvs. Atlas and NM 31

RESULTS AND DISCUSSION

Symptoms appeared 7–10 days after inoculation of seedlings of sweet corn cv. Gold Cup. Symptoms caused by SCMV, MDMV, JGMV, or SrMV in Gold Cup were similar. No visible change in symptoms resulted after the challenge inoculation with the second virus. The presence of the challenge virus was determined by reactions of the sorghum and oat differential cultivars or by serology, or both. The reactions of the differential cultivars to the strains observed were similar to those reported previously (9,30), thus confirming the identity of the inoculated strains.

Among the 53 strain-combinations of the four viruses in the SCMV subgroup tested (Table 1), cross-protection was observed only between SCMV-MDB and SCMV-BC, and only when the former was used as the first inoculated strain and the latter as the challenge strain. No cross-protection was verified when plants infected with SCMV-BC were challenged with SCMV-MDB. Similarly, none of the other combinations of strains of JGMV, MDMV, SCMV, and SrMV resulted in cross-protection.

The one-way protection of SCMV-BC by SCMV-MDB was determined by the reactions of sorghum cvs. Altas and NM 31, which did not produce the specific reactions of SCMV-BC when inoculated with sap from plants doubly inoculated with the two strains. SCMV-MDB induces necrotic streaks and necrotic streaks plus stripes only on the inoculated leaves of Atlas and NM 31, respectively; whereas SCMV-BC induces necrotic streaks and stripes on inoculated as well as new leaves of these two cultivars (30). Although it was not possible

to verify the presence of SCMV-BC serologically due to the unavailability of an antiserum to this strain, the reactions of the sorghum cultivars clearly demonstrated that SCMV-BC did not multiply in Gold Cup plants already infected with SCMV-MDB.

It is generally expected that related strains of the same virus are capable of cross-protection, whereas distinct viruses are not. Therefore, the negative cross-protection results observed with the distinct virus combinations of JGMV, MDMV, SCMV, and SrMV (Table 1) in our investigation are understandable. However, it was surprising to find that, except for the unidirectional protection between SCMV-MDB and SCMV-BC, none of the other 19 combinations involving recognized strains of the same virus, SCMV (Table 1), resulted in cross-protection. Similar results were obtained by Abbott (1), who showed that initially infecting sugarcane plants with SCMV-A did not prevent the multiplication of SCMV-D, and vise versa, after challenge infection.

It is now well established that classical cross-protection is mediated by the viral coat protein (12,20). Comparison of amino acid sequences shows that the surface-exposed N-terminal domain is the only large region in the entire coat protein that is most variable in distinct potyviruses. In contrast, the core and the surface-exposed C-terminal domains are highly conserved (26,32). Therefore, in the case of potyviruses, the N-terminal domain of the coat protein is most likely to be involved in the cross-protection phenomenon, as suggested by Shukla et al (22). This domain has already been shown to mediate aphid transmission (2) and long-distance movement (4) of potyviruses and is thought to influence the host range of potyviruses (33).

Like other potyviruses, comparison of coat protein sequences of JGMV, MDMV, SCMV, and SrMV showed very little similarity in the N-terminal domain but high-level conservation of amino acid residues in the core and C-terminal domains. The sequence identities among the coat proteins of these four viruses comprising the SCMV subgroup has been shown to range from 51 to 71% and the sequence identities among the core proteins of these viruses from 66 to 84% (84 to 88% among MDMV, SCMV, and SrMV) (21). Thus, many of the coat protein sequence differences are attributable to differences in size and sequence of the N-terminal domains, which may be responsible for the negative cross-protection results between strains of these viruses.

In general, strains of individual potyviruses show coat protein sequence identities of more than 90% (26,32). However, when the coat protein sequences of two recognized strains of SCMV, SCMV-SC isolated from sugarcane and SCMV-MDB isolated from maize, were compared, a low-sequence identity of 79% was observed (5). Further examination of the two sequences showed that this low-sequence identity between SCMV-MDB and SCMV-SC is due to an unexpected sequence diversity in the amino-terminal regions of the two coat proteins spanning amino acid residues 27 and 70 in SCMV-SC. This diverse region of SCMV-SC is smaller (44 residues) than the equivalent region in SCMV-MDB (59 residues) and shows only 22% identity to the SCMV-MDB sequence, whereas the rest of the two coat proteins are 92% identical (5).

Recently, amino acid sequences in the variable region of the coat protein N-terminus of five more strains of SCMV (Brisbane, Isis, Sabi, Bundaberg, and BC) isolated from four different plant species were determined. Based on these sequences, the exact size of the hypervariable region in each of the seven SCMV strains was defined. They ranged in length from 21 residues in Bundaberg to 28 in Brisbane, 35 in SC and Isis, 44 in Sabi, 51 in MDB, and 68 in BC and contained repeat sequence motifs (33). Comparison of the sequence identity and the nature of the repeats in the seven sequences revealed five different sequence patterns. These could be grouped into three subsets, which correlated with the host range of the strains. SCMV-Brisbane, SCMV-Isis, and SCMV-SC isolated from sugarcane showed almost identical sequence patterns and formed one subset. The other four strains had different sequence patterns and could be grouped further into a Sabi and Bundaberg subset (strains isolated from Sabi grass and wild sorghum, respectively) and a BC and MDB subset (strains isolated from blue coach grass and maize, respectively) (33). This kind of variation in size and sequence of the N-terminal region has not yet been observed with strains of other potyviruses (27). Thus, it is likely that the unidirectional protection between SCMV-MDB and SCMV-BC and the negative cross-protection results between other SCMV strains are due to sequences in the hypervariable regions of their coat proteins.

Although SCMV-MDB and SCMV-BC formed the same subset based on the sequences in the hypervariable region, the size of this region (51 residues in the former and 68 residues in the latter), and the frequency and pattern of the repeat sequence motifis varied between the two strains. The SCMV-BC sequence contained three copies of the 17 residues repeat, whereas SCMV-MDB had only two. Also, the two strains differed slightly in the pattern of their partial repeat sequence motif (33). These differences may be responsible for the one-way protection between SCMV-MDB and SCMV-BC. Similarly, the negative cross-protection results between SCMV-SC and SCMV-D (both isolated from sugarcane and expected to contain similar sequence motifs) may also be due to the presence of slightly different sequences in their hypervariable regions, as found among SCMV-Brisbane, SCMV-SC, and SCMV-Isis (33). Unfortunately there is no sequence data available for SCMV-D at this time.

The results presented in this paper demonstrate that the strains of JGMV, MDMV, SCMV, and SrMV, previously believed to be strains of the one virus, SCMV, do not cross-protect. Much of the previous conflicting results on cross-protection between strains of viruses in the SCMV subgroup may be attributed to misidentification of the strains used (1,17,19,23,26,28). For example, it was shown that the A and B strains of MDMV do not cross-protect (17,28). Since the B strain of MDMV is now recognized as a strain of SCMV and has been named SCMV-MDB (21), the negative cross-protection results between these two strains neatly conform to their present taxonomic assignments. Similarly, the negative cross-protection reported between SCMV-Jg and SCMV-H (19) can be explained by the fact that these former SCMV strains now represent two distinct potyviruses, MDMV and SrMV, respectively (21). On the other hand, the partial protection observed previously between strains of viruses in the SCMV subgroup (1,19,28,31) may be due to the interference effects resulting from mixed infection of the same plant by two distinct viruses (12). For instance, the multiplication of the challenge virus SCMV-I (now named SrMV-SCI) was found to be much slower in seedlings of sorghum cv. Atlas already infected with MDMV-A than in plants infected with SCMV-I alone, as judged from the distinctive symptoms and recovery of SCMV-I on indicator hosts. This effect was most pronounced when SCMV-I was inoculated 1 or 2 days after MDMV-A (28).

The present results extend these findings and show that negative cross-protection can also result between two strains of the same potyvirus if the N-terminal regions of the coat proteins contain different sequence motifs. Of the 19 strain combinations examined here, only the SCMV-MDB and SCMV-BC pair involved strains known to have the same hypervariable motif. It is not known whether the sequences of SCMV-A, -B, -D, or -Yu conform to the same patterns previously described for SCMV-SC, -Isis, -Brisbane, -Bundaberg, -Sabi, -BC, and -MDB (33). These observations show how the accumulation of point mutations in the genomes of virus strains can have a dramatic impact on their biological properties, such as host range, symptom expression, disease severity, and cross-protection (14).

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