Cross-Protection Against *Bean yellow mosaic virus* (BYMV) and *Clover yellow vein virus* by Attenuated BYMV Isolate M11

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ABSTRACT

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Attenuated isolate M11 of *Bean yellow mosaic virus* (BYMV), obtained after exposing BYMV-infected plants to low temperature, and its efficacy in cross-protecting against infection by BYMV isolates from gladiolus, broad bean (*Vicia faba*) and white clover (*Trifolium repens*) was assessed with western blotting and reverse transcription-polymerase chain reaction. The level of cross-protection varied depending on the challenge virus isolates. Cross-protection was complete against BYMV

isolates from gladiolus, but incomplete against BYMV isolates from other hosts. M11 also partially cross-protected against an isolate of *Clover yellow vein virus*. A comparison of the nucleotide sequence of M11 and those of BYMV isolates from gladiolus and from other hosts showed higher homology among gladiolus isolates than the homology between gladiolus isolates and nongladiolus isolates. In the phylogenetic trees, constructed using the nucleotide sequences of an overall polyprotein of the genomes, five gladiolus isolates clustered together, completely separated from the three BYMV isolates from other hosts. A comparison of the amino acid sequences between M11 and its parental isolate IbG, and analysis of recombinant infectious clones between M11 and IbG revealed that an amino acid at position 314 was involved in the attenuation of BYMV.

Gladiolus (*Gladiolus hybrida*) was introduced in the 1840s to Japan, where it is one of the most popular and economically important perennial floricultural bulb crops (41). A mosaic syndrome caused by *Bean yellow mosaic virus* (BYMV), however, has been a serious problem for gladiolus producers. In our preliminary field survey, gladiolus bulbs were contaminated with BYMV at a high rate. These BYMV-infested bulbs appeared to be an important source of the mosaic syndrome (4,20,22).

BYMV, first reported by Pierce (24), is a member of the genus *Potyvirus* in the family *Potyviridae*, one of the largest plant virus groups. BYMV is found worldwide and causes severe diseases in many leguminous and ornamental plants (2,4,5,13,20). More than 20 aphid species, including *Acyrtosiphon pisum*, *Macrosiphum euphorbiae*, *Myzus persicae*, and *Aphis fabae*, transmit BYMV in a nonpersistent manner (4).

As compared with other potyviruses, BYMV has a relatively wider host range. Many BYMV strains that differ in pathogenicity and serotypes have been reported all over the world (2,13,22,41). On the other hand, *Clover yellow vein virus* (CIYVV), with a host range overlapping that of BYMV, is often confused with BYMV because it is serologically closely related to BYMV and induces inclusion bodies similar to those induced by BYMV in infected cells (2,6,11,13). On the basis of nucleotide sequence information, BYMV and CIYVV are ranked as distinct species in the genus *Potyvirus* (37–39).

Plant viruses in mixed infections are known to interact with each other. For example, in cross-protection, prior infection with one virus (the protective virus) prevents or interferes with sub-

sequent infection by another isolate of the same strain or related strains (the challenge virus) (5,7,9,10,15,16,21). The cross-protection test has been previously regarded as an important means for identifying the same strain or a distinct species of plant virus. However cross-protection studies with BYMV so far have resulted in either complete protection (7,9), partial protection (5,10), or no protection (36). Because of this wide spectrum in previous cross-protection results, we need to reexamine the efficacy of cross-protection among BYMV isolates and related viruses, such as CIYVV.

Cross-protection offers a promising strategy for biological control of plant viruses. We have been successfully developing biological control measures using attenuated virus strains derived from low temperature treatments of infected plants for viruses such as *Soybean mosaic virus*, *Cucumber mosaic virus*, and *Zucchini yellow mosaic virus* (14–17). For BYMV, we obtained a promising attenuated isolate M11 after a low-temperature treatment of a severe isolate originally from gladiolus (21) and developed an efficient method for inoculating mericlone gladiolus seedlings (19) with M11 to prevent the mosaic syndrome on gladiolus.

In this report, we demonstrate the effects of cross-protection by BYMV-M11 against six BYMV isolates derived from gladiolus, broad bean (*Vicia faba*) and white clover (*Trifolium repens*) and show that the level of cross-protection varied depending on the isolate of the challenge virus. Furthermore, by comparing the amino acids that differ between M11 and its parental isolate IbG and using recombination experiments with infectious clones of M11 and IbG, we identified an amino acid that may be involved in the attenuation.

MATERIALS AND METHODS

Viruses and plants. Seven BYMV isolates (M11, IbG, Gla, G1, 90-2, 92-1, and CS), isolate No30 of CIYVV (35), and isolate

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Pk of *Watermelon mosaic virus* (WMV) (1) were used in this study (Table 1). BYMV-IbG, Gla, and G1 were isolated from gladiolus leaves with mosaic symptoms, 90-2 from broad bean (*Vicia faba*), and 92-1 and CS from white clover (*Trifolium repens*). M11 was obtained from BYMV-IbG after a low temperature treatment (21). After three cycles of single lesion isolation on *Chenopodium quinoa*, all isolates were propagated in broad bean. The upper leaves with mosaic symptoms were harvested and stored at -70°C and used for the nucleotide sequencing and the cross-protection experiments.

Sequencing of the viral genome and phylogenetic analysis. To determine the nucleotide sequences of M11, IbG, Gla, G1, 90-2, and 92-1, we extracted total RNA from virus-infected leaves using an RNeasy plant mini-kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. The cDNAs corresponding to the viral RNAs were amplified by reverse transcription-polymerase chain reaction (RT-PCR) as five overlapping sections from the 5'-untranslated region (UTR) to the 3'-UTR using synthetic primers based on the published nucleotide sequences of BYMV (accession D83749 and U47033). The PCR products were purified with the QIAquick PCR purification kit (Qiagen) and were directly sequenced with a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) using walking sequence primers that were designed for every 600 bases. Nucleotide sequence data was compiled and analyzed using the program GENETIX-WIN ver. 6.1 (Software Development, Tokyo, Japan). Sequences were aligned using the program CLUSTAL W, and phylogenetic analysis was done with neighbor-joining method (28) using CLUSTAL W. The tree was drawn with the program Tree View. The nucleotide sequence for BYMV-CS was kindly provided by I. Uyeda (Hokkaido University). The genome sequences for BYMV-GDD (accession AY192568) and CIYVV-No30 (accession AB011819) were also used in the phylogenetic analysis.

Cross-protection experiments. Broad bean seedlings at the three-leaf stage were inoculated with M11 at the second lowest leaf. Eight days after the inoculation (dpi), total proteins were extracted from the inoculated leaf as previously described by Fujii et al. (8) and separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gel was transferred onto a polyvinylidene difluoride (PVDF) membrane and subjected to immunoblotting using a polyclonal antibody against BYMV-90-2 for the infection with M11 (29). The seedlings positive for M11 infection were challenge-inoculated with severe isolates of BYMV or CIYVV on the fourth lowest leaf. WMV-Pk (accession AB218280) was also used as a challenge virus on the control

plants. Upper leaves, seventh or eighth leaf of inoculated plants were tested for systemic infection by the challenge viruses at 10 and 20 dpi with western blotting using either monoclonal antibodies 4H-9 against BYMV-90-2 or 2H-8 against CIYVV-90-1 (30,31), or a polyclonal antibody against WMV-9(M) (12), and with RT-PCR using primer sets specific for each challenge virus (Table 1). Inoculated plants were grown in a greenhouse at 22 to 25°C from March to September. The test had three replications.

Determination of the amino acid involved in the attenuation of BYMV. To ascertain which amino acid was involved in the attenuation of IbG, the recombinants between M11 and IbG were constructed using infectious clones of M11 (pM11) and IbG (pIbG) (33,34). Four restriction sites were used to exchange regions between the infectious cDNA clones in order to construct recombinants (Fig. 1). Each recombinant was first used to inoculate *Nicotiana benthamiana* with a Helios Gene Gun system (Bio-Rad, Hercules, CA) as described previously (33,34). Two weeks later, the systemically infected leaves of *N. benthamiana* were used in mechanical inoculation assays on broad bean seedlings.

RESULTS

Assessment of cross-protection effects by M11 against severe isolates. To assess the level of cross-protection by M11 against severe isolates of BYMV and CIYVV, we used M11 as a protective virus and the severe isolates as challenge viruses to inoculate broad bean plants, then tested infection by the challenge viruses in western blotting and RT-PCR for specific detection of the challenge viruses (Fig. 2). Monoclonal antibodies 4H-9 against BYMV reacted with Gla, G1, 90-2, and CS isolates of BYMV, monoclonal antibodies 2H-8 against CIYVV reacted with CIYVV-No30, and a polyclonal antibody against WMV-9(M) reacted with WMV-Pk, while none of the antibodies reacted with M11 (Fig. 2A). Monoclonal antibody 4H-9 did not react with IbG or 92-1 isolates of BYMV. The serological differences between M11 and the four BYMV isolates and one CIYVV isolate enabled the specific detection of the five challenge isolates in broad bean leaves. In RT-PCR using the primer pairs listed in Table 1, products of expected sizes were amplified from extracts of leaves infected with each challenge isolate, whereas no detectable PCR products from leaves infected with M11 (Fig. 2B). As shown in Table 2, M11 gave complete cross-protection against IbG, Gla, and G1, which were all isolated from gladiolus plants. That is, in broad bean plants preinoculated with M11 and challenge inoculated with IbG, Gla, and G1, the challenge viruses were not detected in western blotting assay and RT-PCR. When plants were

TABLE 1. Original hosts, accession numbers of viral sequences, and primer sequences used to detect a specific sequence (Seq. location) in *Bean yellow mosaic virus* (BYMV), *Clover yellow vein virus* (CIYVV), and *Watermelon mosaic virus* (WMV) isolates used as challenge viruses in this study

Virus isolate	Original host ^a	Accession	Sequence of primer pair	Seq. location
BYMV-M11	<i>Gladiolus hybrida</i>	AB079886	...	...
BYMV-IbG	<i>Gladiolus hybrida</i>	AB079887	Forward: 5'-CTCCAAATGGAGAACCCCGC-3' Reverse: 5'-GATAAGCTCTTTGCCCCAAAC-3'	1370-1697
BYMV-Gla	<i>Gladiolus hybrida</i>	AB439729	Forward: 5'-GGAGCTAGCGATTGGTAGACTC-3' Reverse: 5'-AGTCTGATATAACTGCCGTT-3'	1792-1793
BYMV-G1	<i>Gladiolus hybrida</i>	AB439730	Forward: 5'-GTTAGCTATAGGAAGATTGATC-3' Reverse: 5'-AGTCTGATATGACTGCCGTC-3'	1795-1793
BYMV-90-2	<i>Vicia faba</i>	AB439731	Forward: 5'-GGAATTGCGGTTTTATCTGAC-3' Reverse: 5'-TGCATTGTGCCGAGTTTGTGG-3'	1952-2304
BYMV-92-1	<i>Trifolium repens</i>	AB439732	Forward: 5'-GTTAGCAATTGGTAGGCTCA-3' Reverse: 5'-AATCTGATATAACTGCCGTC-3'	1795-1793
BYMV-CS	<i>Trifolium repens</i>	...	Forward: 5'-ACTTGCCACAGGACGTCTGATAG-3' Reverse: 5'-AATCTGACAGAATAGCTGTC-3'	1795-1793
CIYVV-No30	<i>Phaseolus vulgaris</i>	AB011819	Forward: 5'-TCGTGAGCGTTTAAGTATGG-3' Reverse: 5'-CCTTCAGATGGATCAATCAC-3'	1399-1789
WMV-Pk	<i>Cucumis melo</i>	AB218280	Forward: 5'-GGCAGCAGCAATTAGTCAATC-3' Reverse: 5'-GTATCTGCAGCATATGTGTGC-3'	1582-1863

^a Plant from which the virus isolate was initially isolated.

preinoculated with M11 and then challenge inoculated with 90-2, 92-1, and CS, which were isolated from nongladiolus plants, the cross-protection was partial, and results were similar between western blot and RT-PCR, i.e., RT-PCR detected the challenge viruses, 90-2, 92-1, and CS in plants of 40.0% (6/15), 50% (7/14), and 29% (4/14), respectively. M11 also gave partial cross-protection against CIYVV-No30. Of 11 plants preinoculated with M11 and challenge inoculated with CIYVV-No30, eight plants were systemically infected with the challenge virus. When WMV-Pk was used as a challenge virus, no cross-protection was observed. WMV-Pk was detected in all test plants by western blotting and RT-PCR.

Phylogenetic relationships among BYMV isolates. The nucleotide sequences in the polyprotein region of five BYMV gladiolus isolates, including GDD whose nucleotide sequence was determined in the United States, and three isolates of nongladiolus origin were compared for percent identity at each protein or an overall polyprotein. The five gladiolus isolates, M11, IbG, Gla, G1, and GDD had higher percent identities in the overall polyprotein region, i.e., the percent identity ranged from 92.4 to 99.9% among the five gladiolus isolates and from 75.8 to 87.0% between the gladiolus and nongladiolus isolates, 90-2, 92-1, and CS (Fig. 3A). The five gladiolus isolates had higher percent identities in all polyprotein regions except for the P1, HC-

Fig. 1. Schematic representation of the genome of *Bean yellow mosaic virus*, the structure and pathogenicity of the recombinants constructed by exchanging regions between infectious clones plbG and pM11. Amino acids that differed between IbG and M11 are indicated by arrowheads. Restriction sites used to construct the recombinants and their nucleotide position along the IbG genome are indicated at the top. Summaries of the behavior of plbG, pM11, and their chimeric recombinants on broad bean seedlings are indicated at the right. M = mosaic symptoms; — = no symptoms.

Fig. 2. Specific detection of severe isolates of *Bean yellow mosaic virus* (BYMV), *Clover yellow vein virus* (CIYVV), and *Watermelon mosaic virus* (WMV) by western blotting (A) and reverse transcription-polymerase chain reaction (RT-PCR) (B) in broad bean plants 10 days after challenge inoculation. Monoclonal antibodies 4H-9 against BYMV-90-2 (lanes 1-8) and 2H-8 (lanes 9-11) against CIYVV-90-1, and a polyclonal antibody against WMV-9(M) (lanes 12-14) were used to detect BYMV, CIYVV, and WMV, respectively, in the Western blot analysis, from leaves of broad bean plants infected with M11 (lanes 1, 9, and 12), IbG (lane 2), Gla (lane 3), G1 (lane 4), 90-2 (lane 5), 92-1 (lane 6), CS (lane 7), CIYVV-No30 (lane 10), or WMV-Pk (lane 13) and healthy leaves (lanes 8, 11, and 14). Total RNA from virus-infected leaves of broad bean were subjected to RT-PCR using the specific primers shown above the gel (B). Total RNA from the M11-infected leaves was used as a control in all the RT-PCRs.

Pro, and CP regions than did the other nongladiolus isolates. For example, the percent identity for the CI region ranged from 97.1 to 100% among gladiolus isolates, while it ranged from 78.1 to 89.0% between gladiolus and nongladiolus isolates (Fig. 3B). However, in the case of the CP region, the percent identity ranged from 84.7 to 99.9% among gladiolus isolates and from 82.4 to 91.2% between gladiolus and nongladiolus isolates (Fig. 3C).

Phylogenetic analysis of the nucleotide sequences demonstrated a similar trend as indicated in the analysis of the percent identities. Phylogenetic trees that were constructed on the basis of nucleotide sequences for the overall polyprotein region of the genome indicated that five gladiolus isolates clustered together, completely separate from the three BYMV isolates of nongladiolus origins (Fig. 3A). Also, in the phylogenetic tree constructed on the basis of the CI sequence, five gladiolus isolates formed one cluster and separated from the three BYMV isolates of nongladiolus origins (Fig. 3B). In the phylogenetic tree of the CP sequence, gladiolus isolates M11 and IbG formed one cluster separated from the three other gladiolus isolates Gla, G1, and GDD, which formed another cluster (Fig. 3C).

Amino acid sequences of M11 and an amino acid involved in the viral attenuation. To localize the amino acid involved in the attenuation of BYMV, we determined the complete amino acid sequences of the attenuated isolate M11 and its parental isolate IbG. The genome of both isolates consists of 9,547 nucleotides and contains one large ORF that encodes a polyprotein of 3,056 amino acids. Three amino acids at positions 314, 491, and 856 were differed between the M11 and IbG (Fig. 1).

To determine the amino acid involved in the attenuation of BYMV, recombinant experiments using infectious clones of M11 and IbG were conducted. By using four restriction sites that could exchange the three amino acid residues between M11 and IbG, three infectious recombinant clones, pIbGSB, pIbGBX, and pIbGXP, were constructed (Fig. 1). Recombinant pIbGSB, a pIbG clone was replaced with a region between SphI and BssHIII of pM11 and resulted in the exchange of an amino acid at position 314, was detected from the inoculated broad bean, but it did not induce any symptoms on the plant. In contrast, the other recombinants, pIbGBX and pIbGXP, induced mosaic symptoms similar to those caused by BYMV-IbG (data not shown). Comparison of the amino acid at position 314 between M11 and the five BYMV severe isolates, Gla, G1, 90-2, 92-1, and CS indicated that the amino acid Ser on M11, but not Leu on other five BYMV severe

isolates (data not shown), is critical for the development of mild symptoms thus indicating that the amino acid at position 314 apparently plays an important role in the attenuation of BYMV.

DISCUSSION

It is generally accepted that cross-protection is effective between related strains of the same virus, whereas it is not between distinct viruses. Identities and relationships between two viruses or strains can be presumed based on the occurrence of cross-protection between the viruses or strains. Thus, the cross-protection test has been regarded as an important means for identifying plant virus strains. However, the value of cross-protection data for potyviruses sometimes has not coincided with other taxonomic parameters. For example, unexpected cross-protections between potyviruses previously considered to be distinct were reported between BYMV and each of the following viruses: *Pea mosaic virus*, *Soybean mosaic virus*, and *Bean common mosaic virus* (9,25,44). Unexpected failures in the interaction in cross-protection studies were also observed between mild strains and necrotic strains of *Ryegrass mosaic virus* (42), and between A and B strains of *Maize dwarf mosaic virus* (23). Such results suggested that cross-protection is not an appropriate taxonomic criterion for these viruses (32); however, conflicting results in the cross-protection between potyviruses may be due to technical problems, including lack of specific detection methods for challenge viruses or misidentification of the viruses or strains used. In the present study, six BYMV isolates and one CIYVV isolate used were confirmed as strains of BYMV or CIYVV based on their nucleotide sequence information. The cross-protection observed for BYMV-M11 against the other BYMV isolates were either complete or partial. Furthermore, partial cross-protection was also obtained for BYMV-M11 against CIYVV, a distinct species. These results indicated that cross-protection may not be used for a taxonomic criterion for BYMV and CIYVV, even though cross-protection was previously considered to be very useful as a taxonomic criterion for potyviruses (32).

The mechanisms underlying the cross-protection are not fully understood, however, posttranscriptional gene silencing (PTGS) is considered to be functionally involved in the cross-protection (26,27). PTGS as a defense mechanism against viruses in plants is mediated by virus-derived small interfering RNAs from 21 to 24 nucleotides long (3,18,40). Recently, a computational estimation

TABLE 2. Number of plants of *Vicia faba* positive for challenge virus after preinoculation with attenuated isolate BYMV-M11 to cross-protect against isolates of *Bean yellow mosaic virus* (BYMV), *Clover yellow vein virus* (CIYVV), or *Watermelon mosaic virus* (WMV)^a

Preinoculation	Challenger	Western blot ^b		RT-PCR ^c	
		10 days	20 days	10 days	20 days
BYMV-M11	BYMV-IbG	nt	nt	0/13	0/13
	BYMV-Gla	0/12	0/12	0/12	0/12
	BYMV-G1	0/12	0/12	0/12	0/12
	BYMV-90-2	5/15	5/15	6/15	6/15
	BYMV-92-1	nt	nt	7/14	7/14
	BYMV-CS	4/14	4/14	4/14	4/14
	CIYVV-No30	8/11	7/11	8/11	8/11
	WMV-Pk	6/6	6/6	6/6	6/6
	BYMV-IbG	nt	nt	6/6	6/6
	BYMV-Gla	6/6	6/6	6/6	6/6
Buffer	BYMV-G1	6/6	6/6	6/6	6/6
	BYMV-90-2	6/6	6/6	6/6	6/6
	BYMV-92-1	nt	nt	6/6	6/6
	BYMV-CS	6/6	6/6	6/6	6/6
	CIYVV-No30	5/5	5/5	5/5	5/5
	WMV-Pk	6/6	6/6	6/6	6/6

^a Plants were challenge-inoculated 8 days after preinoculation with BYMV-M11 or 0.1 M phosphate buffer (pH 7.0), then assayed at 10 and 20 days after the challenge for the challenge viruses by western blotting using specific monoclonal or polyclonal antibodies and by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers for the challenge viruses.

^b Number of plants positive in western blotting assay/number of plants tested. nt = not tested; monoclonal antibodies for challenge virus not available.

^c Number of plants positive by RT-PCR/number of plants tested.

system to predict potential targets for PTGS was developed (43). Using this estimation system, we predicted the presence of putative effective small interfering RNA segments in the M11 genome (both negative and positive senses) and analyzed whether corre-

sponding interfering RNA segments exist in the challenge virus genome (*unpublished data*). In fact, numerous interfering RNA segments commonly recognized in the genome of M11 and in each of the challenge viruses were generally consistent with the

Fig. 3. Phylogenetic tree based on the nucleotide sequences of overall polyprotein region (A), CI region (B), and CP region (C) of eight isolates of *Bean yellow mosaic virus* (BYMV). The tree was constructed using neighbor joining, and the numbers on branches are confidence values for 1,000 bootstrap replicates. CIYVV-No30 (accession AB011819) was used as the outgroup. The bar represents a phylogenetic distance of 0.1%.

cross-protection effects observed in this study. With the estimation system for potential RNA segments for PTGS, 876 putative interference RNA segments were recognized in the M11 genome (positive senses). Of 876 RNA segments, 866 were found in the genome of the parental isolate IbG. When M11 and the gladiolus isolates Gla and G1 that M11 completely cross-protected against were compared, 447 and 411 common interference RNA segments were found, respectively, even though G1 was molecularly the most distantly related to M11 among the three gladiolus isolates. In contrast, less than 100 common interference RNA segments were found between M11 and nongladiolus isolates, against which M11 partially cross-protected. The CS isolate, which is the most distantly related to M11, shared only 10 RNA segments. CIYVV-No30, a virus distinct from BYMV but was partially protected against by M11, shared two RNA segments with M11. WMV-Pk, against which M11 did not cross-protect, had no target segments with M11. Similar results were obtained from the analysis for negative sense RNA. These results support the idea that PTGS is involved in the cross-protection.

In the present study, cross-protection using attenuated isolate M11 is one of the most promising strategies to control the mosaic syndrome caused by BYMV in gladiolus. However, we did not use gladiolus for these cross-protection experiments, but used broad bean plants instead. This choice was based on the fact that it is very difficult to show cross-protection effects of M11 against severe BYMV isolates in gladiolus plants, because when compared with broad bean, gladiolus had a low rate of systemic infection with BYMV after sap-inoculation. Furthermore, only young mericlone seedlings of gladiolus were sensitive to BYMV infection (19); thus, systemic infection with BYMV is inconsistent.

In our previous work, BYMV isolates collected from different host plants at different locations in Japan were classified into four pathotypes on the basis of their pathogenicity on four bean cultivars (30). The variability in their biological properties corresponded to some degree to their serological variability (30). Furthermore, biological variability was also correlated with their original host plants. BYMV isolates from broad bean belonged mainly to pathotype II and BYMV from gladiolus to pathotypes I or IV (30). Wada et al. (41) also reported that BYMV isolates from gladiolus differed biologically from nongladiolus BYMV isolates and that their biological variability corresponded to some degree to their serological variability. Biological and serological differences between gladiolus and nongladiolus BYMV isolates were also reported by Nagel et al. (22) in Florida. Phylogenetic analysis of the nucleotide sequences on gladiolus and nongladiolus BYMV isolates presented here strongly supported that the grouping of five gladiolus isolates, including the GDD isolate in the United States, in a cluster separate from the three nongladiolus isolates. The differences in gladiolus isolates from nongladiolus isolates arise from the evolutionary adaptation of gladiolus isolates to its host plant because the gladiolus isolates were most likely maintained through bulb transmission (20). In these experiments, M11, derived from an isolate originally collected from gladiolus, strongly cross-protected against challenge infections by other gladiolus isolates of BYMV and appears promising as a protective virus for controlling the mosaic syndrome caused by BYMV in gladiolus.

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