Pathogenesis of *Alfalfa mosaic virus* in Soybean (*Glycine max*) and Expression of Chimeric Rabies Peptide in Virus-Infected Soybean Plants

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ABSTRACT

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Infection of soybean (*Glycine max*) plants inoculated with particles of *Alfalfa mosaic virus* (AIMV) isolate 425 at 12 days after germination was monitored throughout the life cycle of the plant (vegetative growth, flowering, seed formation, and seed maturation) by western blot analysis of tissue samples. At 8 to 10 days after inoculation, the upper uninoculated leaves showed symptoms of virus infection and accumulation of viral coat protein (CP). Virus CP was detectable in leaves, stem, roots,

seedpods, and seed coat up to 45 days postinoculation (dpi), but only in the seedpod and seed coat at 65 dpi. No virus accumulation was detected in embryos and cotyledons at any time during infection, and no seed transmission of virus was observed. Soybean plants inoculated with recombinant AIMV passaged from upper uninoculated leaves of infected plants showed accumulation of full-length chimeric AIMV CP containing rabies antigen in systemically infected leaves and seed coat. These results suggest the potential usefulness of plants and plant viruses as vehicles for producing proteins of biomedical importance in a safe and inexpensive manner. Moreover, even the soybean seed coat, treated as waste tissue during conventional processing for oil and other products, may be utilized for the expression of value-added proteins.

Infection of alfalfa (*Medicago sativa*) with *Alfalfa mosaic virus* (AIMV) in the United States was first reported in 1931 (32). The virus belongs to the genus *Ilarvirus* and is a member of the family *Bromoviridae*. The genome of AIMV consists of three positive-sense RNAs (RNAs 1 through 3). RNAs 1 and 2 encode replicase proteins P1 and P2, respectively (2), and RNA3 encodes the cell-to-cell movement protein P3 (8,25). The virus coat protein (CP) is translated from the subgenomic RNA4 synthesized from genomic RNA3 (3). AIMV depends on a functional P3 protein for cell-to-cell movement, whereas the CP is required throughout viral infection because of its role in genome activation to initiate the infection (3,30,33,34), RNA replication (6,29), virus assembly (12), stability of viral RNA (13,21), long-distance movement of viral RNA (28), and symptom formation (20). Depending on the size of the CP-encapsidated viral RNA, virions of AIMV can differ greatly in size (30 to 60 nm in length and 18 nm in diameter) and form (spherical, ellipsoidal, or bacilliform).

The host range of AIMV is remarkably wide (7) and includes the agriculturally valuable crops, alfalfa (*M. sativa*), tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), common bean (*Phaseolus vulgaris*), potato (*Solanum tuberosum*), white clover (*Trifolium repens*), and soybean (*Glycine max*). The virus is transmitted by mechanical inoculation or by an aphid vector (7,24) and usually does not persist in the host (4,17,23). Symptoms of virus infection vary from yellow necrotic patterns accompanied by leaf malformations and stunting to formation of a mild, light-green mosaic pattern. Symptoms can either persist or disappear soon after infection, depending on the host plant species and environmental conditions.

Soybean, the most important grain legume crop grown (60 million acres yearly) in the United States, is susceptible to many viruses, including *Soybean mosaic virus*, *Tobacco ringspot virus*, *Bean yellow mosaic virus*, and AIMV. Although the incidence of mosaic diseases is low, diseased plants produce very few seeds and product loss due to infection can be significant (7). In this study, we monitored AIMV pathogenesis and virus accumulation in different parts of greenhouse-grown soybean plants (*G. max*) throughout the entire growth cycle. Systemically infected leaves showed mild mosaic symptoms that persisted throughout the life cycle. Infection also resulted in stunted growth and decreased seed production. In spite of some reduction in growth and seed production, virus-infected soybean plants can be an excellent source for value-added proteins. Inoculation of soybean plants with recombinant virus containing rabies peptide resulted in the production of full-length chimeric protein that reacted with antibodies specific for AIMV CP and surface glycoprotein of rabies virus.

MATERIALS AND METHODS

Inoculum preparation. Purified AIMV isolate 425 was provided by S. Loesch-Fries (Purdue University, Lafayette, IN). Stock virus was prepared from the leaves of *Nicotiana tabacum* cv. Xanthi-nc plants inoculated with AIMV virions diluted in FES buffer (sodium-pyrophosphate [1%, wt/vol], macaloid [1%, wt/vol], celite [1%, wt/vol], 0.5 M glycine, and 0.3 M K₂HPO₄, pH 8.5, with phosphoric acid). Virus was isolated as described (35) at 15 to 20 days after inoculation. Briefly, leaf tissue was ground and the sap separated from cell debris by centrifugation. Virus particles were selectively precipitated with 5% polyethylene glycol. Purified virus was used to inoculate soybean plants.

In vitro transcripts of AIMV RNAs were synthesized using T7 (for genomic RNAs 1, 2, and 3) or SP6 (for subgenomic RNA4) RNA polymerases (Promega, Madison, WI) and purified plasmid DNA, according to the manufacturer's guidelines. Purified

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plasmid DNAs corresponding to genomic RNAs 1, 2 (pUT17 and pUT27, respectively) (19), and 3 (recRNA3), or subgenomic RNA4 (pSP65A4) (13) of AIMV were linearized with *Sma*I at the 3' end of the viral RNA sequences before the in vitro transcription reaction. The reaction was performed in a 100- μ l volume. Transcripts were capped with the RNA cap structure analog m7G(5)ppp(5)G (Biolabs, Beverly, MA).

Plant growth and infection. Seeds of *N. tabacum* cv. Xanthi-nc, *N. benthamiana*, and *G. max* (obtained from R. Muse, Delaware Valley College, Doylestown, PA) were germinated and maintained in the greenhouse for the entire time of study. *N. tabacum* cv. Xanthi-nc and *N. benthamiana* plants were inoculated with AIMV diluted in FES buffer (2 ng/ μ l) (31) by application on-to four upper leaves (at six-leaf stage) abraded with Carborundum (320 grit; Fisher, Pittsburgh, PA), followed by gentle rubbing to spread the inoculum and further abrade the leaf surface. Cotyledons and two leaves of 12-day-old soybean seedlings were inoculated with AIMV diluted in FES buffer (2 ng/ μ l) (31). Experiments with soybean plants were performed twice (February through April and September through November 2000) using three separate batches of plants (20 plants per batch) inoculated at 1-week intervals in each experiment. Virus was isolated from leaves, roots, and seed coats of infected soybean plants (35).

For infection with recombinant AIMV containing chimeric peptide representing regions from rabies virus glycoprotein and nucleoprotein, soybean plants were inoculated with a mixture of in vitro-synthesized transcripts of AIMV genomic RNAs 1, 2, and 3, and subgenomic RNA4. Genomic RNA3 contained nucleotide sequences encoding the rabies peptide, which was cloned and expressed as an in-frame, N-terminal fusion with AIMV CP. To assess the stability of recombinant virus, we prepared extracts from systemically infected leaves of plants (inoculated with a mixture of in vitro synthesized transcripts) at 20 days post-inoculation (dpi) and used it as an inoculum for healthy seedlings.

Sample preparation and western blot analysis. Tissue obtained from leaves, roots, seedpods, seeds, seed coats, cotyledons, and embryos were placed in Eppendorf tubes, frozen in liquid nitrogen, ground, and boiled for 10 min in 2 $\times$ loading buffer (2% sodium dodecyl sulfate [SDS], 10% glycerol, 5% β -mercapto-

ethanol, 0.01% bromophenol blue, and 50 mM Tris-HCl, pH 6.8). After centrifugation, supernatants were analyzed for the presence of AIMV CP by western blotting (35). Briefly, proteins were separated electrophoretically on SDS-polyacrylamide gels and electro-blotted onto a nitrocellulose membrane for 1 h at 100 mA. After blocking with 5% dry milk, proteins were reacted with monoclonal antibodies against AIMV CP conjugated with horseradish peroxidase (Agdia, Elkhart, IN) and detected by the ABC kit (Vector Laboratories, Burlingame, CA) or chemiluminescent substrate (Boehringer, Indianapolis, IN). Recombinant AIMV CP containing chimeric rabies peptide, in addition, was detected with rabies virus G protein-specific antibodies (35).

To prepare root samples, at 45 dpi, AIMV infected or equivalent control soybean plants were removed from their pots, placed in a tray with distilled water, and washed by shaking at room temperature. The washing continued until all soil was removed. After washing was complete, the residual water was removed with paper towels, and roots were used for further experimentation.

To assess the accumulation of virus in leaves at particular times, leaf samples (one leaf per plant at each time) were obtained from the whole batch (20 plants per batch). The leaf was taken from the second or third internode from the apex.

Statistics. The average length of internodes in AIMV-infected and healthy plants was calculated at 45 dpi. Measurements were taken from three separately infected batches of plants (20 plants per batch). Five plants in each batch were sacrificed to calculate the root mass. The virus yield was averaged from three separate isolations. Standard deviations and *t* test (*P*) were calculated using Microsoft Excel version 5.0a for Power Macintosh.

RESULTS

Symptoms of AIMV infection. Soybean plants showed mosaic symptoms of AIMV infection and yellowing in the upper uninoculated leaves within 10 to 12 dpi (Fig. 1A). These symptoms persisted throughout the life cycle of the plant. In some of the older leaves, the mosaic pattern changed to mold-like symptoms, particularly in the vicinity of main leaf veins (Fig. 1B). Infection resulted in significant stunting of plant growth (Fig. 2), with

Fig. 1. Symptomatology of *Alfalfa mosaic virus* infection in soybean plants. **A**, Accumulation of virus in systemically infected leaves induces mosaic patterns and yellowing (arrows). **B**, In older leaves, the mosaic develops into mold-like symptoms particularly noticeable along the leaf veins (arrows).

average length of internodes in AIMV-infected plants calculated at 45 dpi, less than half that of control-uninoculated plants (5.8 ± 0.6 cm versus 14.2 ± 0.6 cm; $P = 0.0001$) of the same age. Although the length of internodes in infected plants was dramatically reduced, the number of internodes did not change dramatically (5 ± 1 versus 7 ± 1 ; $P = 0.0350$, data collected at 45 dpi). Root mass in infected plants was dramatically reduced compared with that of healthy control plants (2.8 ± 0.8 g versus 9.5 ± 1.5 g; $P = 0.0002$), and infection decreased leaf size, although the shape of leaves was the same in infected and control plants. In both experiments, symptoms of virus infection were similar, however, there were some differences in the time of flowering and seedpod formation. Whereas plants inoculated in the spring (February through April) flowered and formed seedpods at essentially the same time as did healthy plants, the number of seedpods formed per infected plant was less than that of control plants (20 ± 3 versus 30 ± 2 ; $P = 0.004$). Plants infected in the fall (September through November) showed a 2-week delay in flowering and pod formation compared with that of healthy control plants. Infection did not significantly affect the number of seeds per pod and mature soybeans harvested from AIMV-infected and control plants were morphologically indistinguishable in both experiments. Together, these findings indicate that symptoms of AIMV infection persist throughout the life cycle of the plant, resulting in significant stunting of growth (reduced internode length), limited root formation, and decreased seed production (up to 35%). It should be emphasized, however, that when we decreased the amount of virus in the initial inoculum (0.5 ng/ μ l), the onset of systemic symptoms was delayed and the severity of stunting of plant growth was reduced. Seed production was reduced only up to 25%. Even so, it did not significantly affect overall accumulation of virus in different parts of the plant and time of virus movement from locally inoculated into the upper uninoculated leaves (data not shown).

AIMV CP accumulation in infected plants. Western blot analysis of tissue samples from locally and systemically infected leaves collected at 15 dpi revealed a band of the size expected for the AIMV CP (24.0 kDa), as detected by reactivity with AIMV CP-specific antibodies (Fig. 3A). Similar analysis of a second set of tissue samples from systemically infected leaves and newly forming seedpods collected at 25 dpi also revealed AIMV CP in both tissue types but not in samples from healthy plants (Fig. 3B). By 35 dpi, AIMV CP was detected in fresh beans, in addition to systemically infected leaves and seedpods (Fig. 3C). At 45 dpi, AIMV CP was detected in samples obtained from systemically infected leaves, seedpod, and seed coat (Fig. 3D). At 55 dpi, CP levels in leaf tissue were noticeably decreased (Fig. 3E) and at 65 dpi undetectable (Fig. 3F). Only seedpods and seed coats contained AIMV CP at day 65 (Fig. 3F). Neither cotyledons nor embryos (dissected from five seeds) contained detectable AIMV CP at any time analyzed. In addition, inoculation of *N. benthamiana* plants with extracts either from cotyledons or embryos never resulted in infection, consistent with the results of western analysis, whereas plants inoculated with extracts from leaves, seedpods, or seed coat all showed AIMV-specific symptoms. In seeds collected from AIMV-infected plants and stored at 4°C for 1 year, only seedpods and seed coats contained detectable AIMV CP (data not shown). *N. benthamiana* plants inoculated with extracts of seedpods and seed coats exhibited symptoms of virus infection in both inoculated and upper uninoculated leaves at 10 dpi (data not shown), indicating that the storage period did not dramatically affect AIMV infectivity.

Western analysis of root samples from infected soybean plants revealed the 24.0-kDa protein as detected by reactivity with AIMV CP-specific antibodies (Fig. 4, root-1 and root-2). Similar analysis of roots that had been washed, air dried, and stored at room temperature for 2 weeks also revealed reactivity with the 24.0-kDa protein (Fig. 4, dry-1 and dry-2), indicating the stability

of AIMV CP in dried root material. Moreover, *N. benthamiana* plants inoculated with extracts from dried roots showed AIMV-specific symptoms in locally inoculated and upper uninoculated leaves. Levels of AIMV in systemically infected leaves began to decline after 30 days (0.9 ± 0.11 , 0.5 ± 0.07 , and 0.2 ± 0.025 mg of AIMV recovered per gram of fresh leaf tissue at 30, 45, and 55 dpi, respectively), whereas the amount of virus recovered from seedpod (0.6 ± 0.09 mg/g of fresh tissue) and seed coat (0.93 mg/g of fresh tissue; material combined from three experimental batches) remained high even at 70 dpi. AIMV could be recovered from roots at 65 dpi (0.15 ± 0.03 mg/g of root tissue) by the standard procedure used to isolate AIMV from leaf tissue.

Seedlings germinated from virus-infected plants contained no detectable AIMV, consistent with the absence of detectable virus in viable embryos or cotyledons. CP-positive seedpods and seed coats do not become a part of newly germinated seedlings and, thus, do not normally transmit virus to seedlings, which is in agreement with early reports (24).

Cloning and expression of foreign sequences fused with AIMV CP in soybean plants. Chimeric peptide, representing rabies virus glycoprotein (amino acids 253-275) and nucleoprotein (amino acids 404-418), which induce protective immunity in animals (35), is an excellent candidate for subunit vaccine development. DNA sequences coding for 38 amino acid peptide were polymerase chain reaction (PCR) amplified using two primers, with 15 overlapping nucleotides that served as a template for each other. The resulting PCR product was digested utilizing artificially created restriction sites (*Xho*I at 5' and *Sal*I at 3' ends) and cloned into the open reading frame of AIMV CP in pSPΔAUG (33) using an *Xho*I site. After sequence confirmation, the chimeric AIMV RNA4 containing sequences encoding rabies peptide was introduced into full-length infectious RNA3 (Fig. 5) that was utilized as an expression vector. Translation of recombinant CP in recRNA3 (Fig. 5) starts from the ATG codon introduced at the 5' end of the DNA sequences encoding the chimeric rabies peptide.

Soybean plants were inoculated with the mixture of in vitro-synthesized transcripts of recRNA3 (containing coding sequences

Fig. 2. Comparison of Alfalfa mosaic virus-infected soybean plants and non-infected control plants (healthy). Note the stunted growth of the infected plant.

for AIMV CP fused with the 38 amino acid peptide from rabies virus), RNA1, RNA2, and RNA4. At 10 to 12 dpi, mild mosaic symptoms of virus infection were observed in the upper uninoculated leaves. Infection with the recombinant virus resulted in stunting of plant growth similar, but not as severe, to that observed after wild-type AIMV infection. Western blot analysis of infected leaf samples at 15 dpi demonstrated expression of recombinant protein in the systemically infected leaves, with antibodies specific for AIMV CP (Fig. 6A) and for rabies virus glycoprotein (Fig. 6B) revealing the expected size protein (29.3 kDa). Wild-type AIMV CP reacted only with AIMV CP-specific antibodies and not with antibodies specific for rabies virus glycoprotein (Fig. 6A and B). After western blot analysis, extracts from systemically infected leaves of these plants were used to inoculate a new batch of healthy soybean seedlings. At 30 and 70 dpi (time when the optimum amount of fresh seed coat tissue containing highest quantity of virus can be recovered), the recombinant virus was isolated from systemically infected leaves (0.5 mg/g of fresh tissue) or seed coat (0.62 mg/g of fresh tissue) of newly infected plants, respectively, and assessed by western blot analysis (Fig. 6C). The optimal time of recombinant virus purification was determined based on our observations of AIMV accumulation in infected soybean plants. As shown in Figure 6, panel C, virus isolated from both leaves and seed coat contain expected size protein (29.3 kDa) that reacts with AIMV

CP-specific antibodies. Recovery of virus consisting of the full-size recombinant protein from systemically infected leaves and seed coat, after virus was passed from plants inoculated with in vitro-synthesized transcripts, demonstrated the stability of the engineered construct and usefulness of soybean as production host. Recombinant protein was also detected in roots and seed-pods of infected plants (data not shown), suggesting the potential usefulness of each of these organs for producing value-added proteins.

DISCUSSION

Knowledge of the disease process induced by plant pathogens in a particular host is a first step toward developing appropriate control measures, including the introduction of pathogen-resistant traits (1). This information is also useful when developing viral vectors for foreign gene expression, because the location and the levels of virus accumulation will often reflect the location and levels of foreign protein accumulation.

Information about the pathogenicity of this virus in host species other than *M. sativa* has been limited. We studied the susceptibility of soybean plants to AIMV infection under greenhouse conditions, as well as the overall effects of AIMV-induced disease on plant growth, flowering, and seed production. Greenhouse-grown soybean plants were highly susceptible to virus infection

Fig. 3. Western blot analysis of coat protein (CP) expression in soybean plants infected with wild-type *Alfalfa mosaic virus* (AIMV). Proteins were separated electrophoretically on a 12% sodium dodecyl sulfate-polyacrylamide gel, transferred to a membrane, and reacted with monoclonal antibodies specific for AIMV CP. Antibodies recognized the expected size protein (24.0 kDa) in samples collected on **A**, 15 and **B**, 25 days postinoculation (dpi) from locally (L) inoculated and systemically (S) infected leaves as well as in newly forming seedpods. Extracts from uninoculated control plants (Healthy) showed no product. **C**, Samples obtained from systemically infected leaf, seedpod, and seed at 35 dpi all revealed AIMV CP. **D to F**, Samples were obtained from systemically infected leaf, seedpod, seed coat, cotyledon, and embryo at 45, 55, and 65 dpi, respectively; AIMV CP was detected at each time point in samples from seedpod and seed coat, and up to 55 dpi in samples from leaf tissue. AIMV CP was not detected in samples from cotyledon and embryo. In all panels, AIMV CP from purified virus was used as a positive control.

during the first 2 to 3 weeks after germination. Within 8 to 10 days after inoculation, virus moved to the upper uninoculated leaves, establishing systemic infection that persisted, in some parts of plants, throughout the life cycle of the plant and accumulated in leaves, roots, seedpod, and seed coats. The spread of virus resulted in mild mosaic symptoms in systemically infected leaves without additional leaf deformation. However, the infection caused stunting of plant growth, limited root formation, and decreased seed production (up to 35%).

Some plant RNA viruses do not replicate continuously in all host species, and infection may not persist throughout the life cycle of the plant. For example, in AIMV-infected tobacco plants, virus concentrations are highest at 15 to 20 days after inoculation, declining and even clearing thereafter (23). Our soybean plants supported high levels of AIMV replication in leaf tissue up to 30 days before a gradual decrease. Only after 65 days (mature seed stage) was AIMV CP no longer detected in leaf samples by western blot analysis. However, significant quantities of virus

were detected in seed coat and seedpod even after 1 year of storage at 4°C, and the virus remained infectious as observed in *N. benthamiana* plants. No morphological differences between seeds from healthy and AIMV-infected plants were observed, except in very few seeds that had deformed seed coats.

In recent years, plants have been actively targeted for the production of medically important proteins, including vaccine antigens and monoclonal antibodies (11). A variety of antigens have been expressed in transgenic plants, including hepatitis B surface antigen (15), *Escherichia coli* heat-labile enterotoxin (10), rabies virus glycoprotein (16), and Norwalk virus capsid protein (14). In addition to transgenic plants, viruses that infect plants have been used as an alternative approach to express high levels of foreign proteins (5,9,18,22,26,27,35). An important aspect of using plant viruses as production tools is screening and establishing appropriate host species for manufacturing large quantities of polypeptides. Depending on the specific product and target population, it is important to use the appropriate plant species with targeted expression of the product in different parts of the plant (leaves, roots, or seeds). The present study suggests the usefulness of soybean plants as a vehicle for producing biomedical reagents utilizing an AIMV-based expression vector. If the leaf tissue is the source of biomedical, a period of 30 days is sufficient to obtain the highest accumulation of virus at maximum yield of biomass. Alternatively, roots, seeds, or seedpods can be targeted as the source of product and can be stored and distributed relatively easily. In fact, because the virus accumulates in readily separated seed coat, this portion of the plant might provide a rich source of value-added proteins. Indeed, we have demonstrated that sufficient quantities of recombinant virus containing chimeric rabies peptide can be recovered from systemically infected leaves (0.5 mg/g of fresh tissue) at 30 dpi and from seed coat (0.62 mg/g of fresh tissue) at 70 dpi. In addition, rabies/foreign sequences fused to AIMV CP can be recovered in a very cost-effective manner utilizing standard virus isolation procedure. Introduction of foreign sequences, however, have resulted in some attenuation of virus accumulation and severity of symptoms. For example, the yield of recombinant AIMV compared with that of wild-type AIMV in systemically infected leaves (0.9 ± 0.11 mg/g of fresh tissue) at 30 dpi or seed coat (0.93 mg/g of fresh tissue) at 70 dpi is 35 to 40% lower. Reduction of symptom severity, however, results in the accumulation of larger amounts of source material (leaves, roots, seedpod, and seed coats). Thus, considering the quantities of virus we are able to recover, utilizing a very economical procedure (virus isolation), this approach has great potential for producing foreign sequences in soybean plants in a safe, time- and cost-efficient manner.

Fig. 4. Western blot analysis of coat protein (CP) expression in roots of soybean plants infected with wild-type *Alfalfa mosaic virus* (AIMV). Proteins were separated electrophoretically on a 12% sodium dodecyl sulfate-polyacrylamide gel, transferred to a membrane, and reacted with monoclonal antibodies specific for AIMV CP. Antibodies recognized the expected size protein (24.0 kDa) in samples from fresh roots (root-1 and root-2) as well as in root tissue dried and stored at room temperature (dry-1 and dry-2). Antibodies did not react with any proteins in root extracts from control (Healthy) plants. AIMV CP from purified virus was used as a positive control.

Fig. 5. Schematic representation of *Alfalfa mosaic virus* RNA3 (recRNA3) encoding a coat protein (CP) gene fused with the sequences encoding chimeric rabies peptide. P3, cell-to-cell movement protein. Arrow indicates the subgenomic promoter for RNA4. 31D is a peptide (T cell epitope) from rabies nucleoprotein. g24 is a peptide (B cell epitope) from rabies virus glycoprotein. Amino acid sequences of the rabies peptides are indicated in the text.

Fig. 6. Western blot showing accumulation of recombinant *Alfalfa mosaic virus* (AIMV) coat protein (CP) (Cpg24) fused to rabies virus peptide in **A and B**, systemically infected soybean leaves, and **C**, virus samples isolated from systemically infected leaves or seed coat. **A and B**, Leaves were inoculated with in vitro-synthesized transcripts of recombinant virus. At 10 to 12 days postinoculation (dpi), samples from systemically infected leaves were collected, and proteins were separated by electrophoresis on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and reacted with monoclonal antibodies specific for **A**, the AIMV CP, or **C**, the linear epitope (G5-24) of rabies virus glycoprotein (R-Gp, purified rabies virus glycoprotein 60 kDa). **C**, Plants were inoculated with extracts passed from systemically infected soybean leaves. Recombinant virus was purified from systemically infected leaves at 30 dpi [CPg24(L)] and seed coat at 70 dpi [CPg24(SC)]. Samples of purified virus were separated by electrophoresis on a 12% SDS-polyacrylamide gel and reacted with monoclonal antibodies specific for the AIMV CP, revealing expected size (29.3 kDa) protein in preparations from both leaves and seed coat. Wild-type AIMV CP (24 kDa) reacted only with **A and C**, antibodies against AIMV CP, and **B**, not with antibodies against rabies peptide. **C**, Extracts from healthy plants did not react with antibodies against AIMV CP.

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