A New Cowpea Strain of Southern Bean Mosaic Virus from Ghana

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Accepted for publication 8 March 1974.

ABSTRACT

A seed-transmissible virus isolated from seedlings of cowpea produced in Ghana was identified as a strain of southern bean mosaic virus (SBMV). The virus infects both cowpea (*Vigna unguiculata*) and bean (*Phaseolus vulgaris*) systemically, but no systemic symptoms are produced in bean. The virus is antigenically related, but not identical to, SBMV and severe bean mosaic virus (SvBMV) on the basis of immunodiffusion with homologous and heterologous sera. Electrophoretic mobilities of -3.3×10^{-5} and -3.7×10^{-5} cm^2 sec^{-1} $volt^{-1}$ at pH 7.0 were calculated for the virus and SBMV by sucrose gradient electrophoresis. The possible derivation of SBMV and the cowpea strain of SBMV (SBMV-CS) from the Ghana strain of SBMV is discussed.

Phytopathology 64:1100-1104

References to viruses of cowpea in Ghana have been few and very general without specification as to the type of symptom, strain of virus involved, or cowpea varietal reaction (14). A seed-borne mosaic virus which was observed to be widely distributed in Ghana was isolated from Ghana cowpea seeds germinated in a greenhouse.

In preliminary studies, the virus associated with the mosaic symptom was identified as a strain of southern bean mosaic virus (SBMV) based on a positive serological reaction with SBMV antiserum (13). The virus was also

found to infect bean in addition to cowpea. It was provisionally named SBMV-GH and is referred to in this report as GH. Except for severe bean mosaic virus (SvBMV) which has been reported to infect both bean and cowpea (29), neither the bean nor the cowpea strains of SBMV has previously been reported to infect reciprocal hosts (24). Work presented here was undertaken to determine the relationship between GH and SBMV and to determine which tissue of the seed was associated with seed transmission.

MATERIALS AND METHODS.—*Viruses.*—GH was isolated at Macdonald College, Quebec, from seedlings of cowpea (*Vigna unguiculata*) grown from seed produced in Ghana. The virus was maintained in Early Ramshorn cowpea after local lesion passage through Georgia 21 cowpea. SBMV (A.T.C.C. No. 17) and a culture of SvBMV, obtained from R. J. Shepherd, Department of Plant Pathology, University of California, Davis, were maintained in French bean (*Phaseolus vulgaris* 'Bountiful').

Host range.—Forty species and varieties of plants which comprised 39 genera in nine families were inoculated with the GH isolate. Selection of the plant species was made to include those reported to be hosts for both the cowpea (SBMV-CS) and bean strains of SBMV. Extracts from all inoculated plants were assayed for GH infectivity on primary leaves of an unknown variety of cowpea (unidentified as to name or plant introduction number) obtained from J. D. Gay, USDA, ARS, Tifton, Ga. This plant was the standard local lesion host for all local lesion assays of GH reported here. All inoculations were made on 10- to 12-day-old plants.

Seed transmission.—Studies on seed transmission in cowpea were carried out by assaying for the presence of GH in flowers, seeds, and seedlings.

Flowers from GH-infected Early Ramshorn cowpea were harvested a day after opening and aseptically separated into their component parts, viz. sepals, petals, pistil, and stamens. The parts were decontaminated with detergent (22), homogenized in 0.01 M phosphate buffer (pH 7.1) and inoculated to the cowpea local lesion host.

For detection of seed-borne virus, seeds were obtained from pods harvested at the following four developmental states: (i) very dark green pods which were relatively immature and which contained rapidly developing green seeds; (ii) pale green pods which had attained full size with relatively mature seeds and which lacked chlorophyll in either seed coats or embryos; (iii) pods which were at the yellow stage but prior to drying and which contained mature seeds; and (iv) mature seeds from very dry pods which had been stored from 1 to 6 mo at room temp.

Seeds were dissected into embryos and seed coats. Pooled embryos as well as seed coats were divided into two lots and weighed. One lot was washed with detergent followed by rinsing under running tap water for 30 min, while the other remained unwashed. Seeds that had been stored were presoaked for 5 min before dissection. Each lot of seed parts was triturated in an equal weight of 0.01 M phosphate buffer (pH 7.1) and inoculated to the cowpea local lesion host.

Seedling tests for detection of seed transmission were carried out by germinating mature seeds from infected plants. Seedlings which emerged were scored by visual examination and by the microprecipitin test for the presence of virus.

Virus purification.—Systemically infected leaves of Early Ramshorn cowpea inoculated 10 to 14 days earlier with GH served as the source of virus. Infected tissue was frozen until used.

The virus was purified by thawing and homogenizing a known quantity of infected tissue in an equal volume of 0.1 M phosphate buffer (pH 7.0) containing 0.1 M ascorbic acid (6). After clarification of the crude extract by low-speed centrifugation (12,000 *g*), the extract was made 10% with chloroform-butanol (1:1) and stirred for 30 min. The mixture was subjected to low-speed centrifugation, and the partially clarified supernatant fluid was given three differential centrifugations (12,000 *g* and 105,651 *g*). The pellets after differential centrifugations were dissolved in 0.01 M phosphate buffer, pH 7.1. Six ml of the virus suspension were floated on 2 ml of 45% sucrose and given a high-speed centrifugation (218,010 *g*) for 4 h. The final pellet (purified virus) was dissolved in a known quantity of 0.01 M phosphate buffer pH 7.1 and stored at 3 C.

Virus characterization.—The thermal inactivation point of GH in sap (diluted 10-fold with distilled water) was determined by infectivity assay of 2-ml aliquots previously heated for 10 min at 5 C intervals from 45-95 C. Infectivity dilution end point was determined by infectivity assay of aliquots of sap (diluted 1:1 with distilled water) serially diluted 10-fold to 10^{-12} with distilled water.

The sedimentation coefficient was determined for purified virus by sucrose gradient centrifugation using tobacco mosaic virus as an internal standard (2). Analytical gradient centrifugation (3) of GH, SBMV, and a physical mixture of both, was also used to estimate the sedimentation coefficient of GH. In both experiments, the amounts of virus centrifuged were determined using an extinction coefficient of 5.85, the reported value for SBMV (8); previous absorbance measurements had established that the ultraviolet absorbance spectra of GH and SBMV were identical.

Sucrose-gradient electrophoresis of GH and SBMV was done in an ISCO model 210 density-gradient electrophoresis apparatus (4) using a 5-20% sucrose gradient buffered at pH 7.0 with 0.01 M Na_2HPO_4-0.005 M citric acid. Each electrophoresis run was for at least 100 min at a constant current of 3 mA. Mobilities were determined by the method of Ball (1).

Serological properties of GH were determined using rabbit antisera to three strains of SBMV. Antiserum to GH was prepared by four weekly intramuscular injections of 2.5 mg of purified virus. Serum was mixed with an equal volume of glycerine and stored at 3 C until used. Serum titers were determined by the microprecipitin method (28). Antisera to SBMV and SvBMV were generous gifts from J. G. McDonald, Department of Plant Pathology, Macdonald College, and R. G. Grogan, Department of Plant Pathology, University of California, Davis, respectively. Antigenic differences between viruses were determined by the microprecipitin and gel-diffusion methods. Conventional Ouchterlony (19) gel diffusion was employed with 1% Noble agar gels buffered at pH 7.1 with 0.01 M potassium phosphate containing 0.14 M

NaCl and 0.02% sodium azide. Serum was deposited in a central well and antigens were deposited in peripheral wells; the distance, center to center, between all wells was 1.2 cm. Intragel cross-absorption studies (27) employed the same gel pattern. The central well was filled initially with purified virus which was allowed to diffuse for 24 h; it was then charged with heterologous antiserum and the peripheral wells were charged with the appropriate antigens. Immunoelectrophoresis was done in agar gels on glass microscope slides (21). The gels were buffered at pH 7.5 with 0.01 M potassium phosphate and 0.04 M NaCl. Antigens were dialyzed for 48 h against the electrolyte. An LKB 6800 A electrophoresis apparatus was used; electrophoresis was for 2 h at 4 C, with a constant current of 26 mA.

Purified virus (0.1 mg/ml) was examined in the electron microscope using the negative stain method. Equal volumes of virus and 2% phosphotungstic acid adjusted to pH 7.0 with 2N NaOH were mixed and examined after drying with an AEI EM 6B electron microscope.

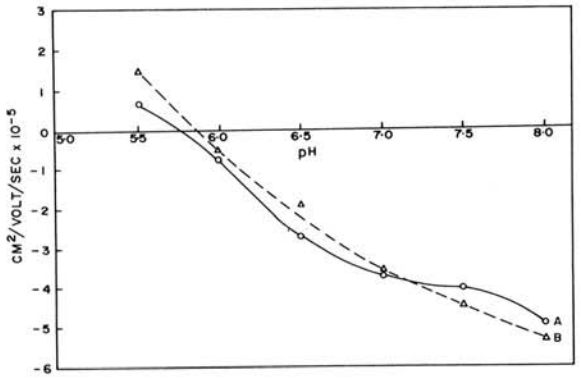

Fig. 1-(A, B). Relationship between pH and electrophoretic mobility of southern bean mosaic virus (SBMV) and the Ghana isolate of southern bean mosaic virus (GH). **A)** SBMV O---O; **B)** GH △...△.

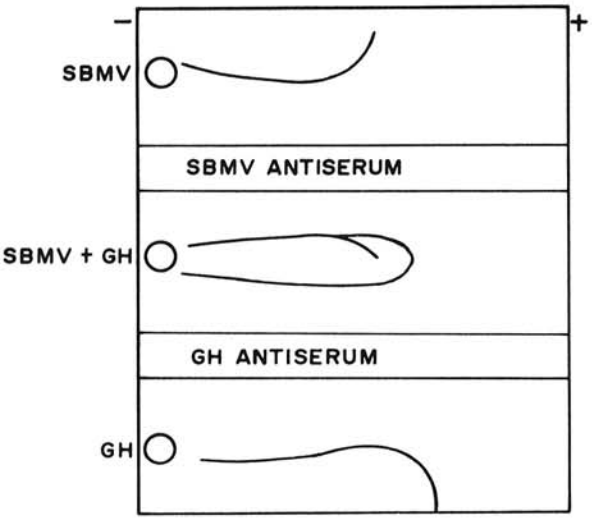

Fig. 2. Immunoelectrophoresis in agar-gel of southern bean mosaic virus (SBMV), the Ghana isolate of southern bean mosaic virus (GH) and an in vitro mixture of both. Electrophoresis was in 0.01 M phosphate, 0.04 M NaCl pH 7.5 for 300 min with a current of 26 mA at 250 V.

RESULTS.—*Host range and symptomatology.*—Inoculation of GH to different hosts indicated that the host range of the virus was restricted to the Leguminoseae. Inoculated primary leaves of Early Ramshorn cowpea showed chlorotic local lesions followed by systemic invasion of developing leaves. Vein clearing was evident in the young trifoliolate leaves. In mature leaves, light- and dark-green mosaic patterns were produced and the leaves became distorted and greatly reduced in size. Stunting was evident; flower fall was prominent and seed set was very poor. Necrotic local lesions were produced on inoculated primary leaves of *Phaseolus aureus* Roxb.

Despite repeated attempts, the following hosts remained symptomless after the inoculation and no virus was recovered: *Amaranthus retroflexus* L., *Arachis hypogaea* L., *Brassica chinensis* L., *Cajanus cajan* (L.) Millsp.; *Capsicum frutescens* L. 'Little Beauty'; *Carnivalia ensiformis* (L.) DC.; *Chenopodium alba* L., *Chenopodium amaranticolor* Coste and Reyn; *Chenopodium quinoa* Willd; *Circer arietinum* L.; *Coronella varia* L. 'Emerald'; and *Crotolaria spectabilis* Roth; *Cucumis sativus* L.; *Datura stramonium* L.; *Dolichos lablab* L.; *Glycine max* (L.) Merr. cv. Fiskeby, Mint and Altona; *Helianthus annuus* L.; *Lactuca sativa* L. 'Meikoningen'; *Lens esculenta* Moench.; *Lotus* corniculatus L. 'Leo'; *Lycopersicon esculentum* Mill; *Medicago sativa* L.; *Nicotiana tabacum* L. 'White Burley'; *Nicotiana glutinosa* L.; *Onobrychis sativa* Lam. 'Eski', *Petunia hybrida* Ulm. 'Nana Compacta'; *Phaseolus lunatus* L. 'Henderson Bush'; *Pisum sativum* L. 'American Wonder'; *Trifolium pratense* L.; *Trifolium repens*; *Vicia faba* L. 'Madagascar Periwinkle'; and *Zinnia elegans* Jacq.

Varietal reactions.—Inoculation of GH to different varieties of cowpea and French bean revealed differences in varietal reactions. The French bean cultivars Highlander, Tendergreen, Slendergreen, Romano, and Black Valentine appeared to be immune; no virus was recovered from them. Chlorotic local lesions on inoculated primary leaves were obtained in Bountiful, Contender, Light Red Kidney, and Logan; these hosts became systemically infected but they were symptomless carriers. The cultivars Pinto, Early Blue Lake, and Kentucky Wonder Wax produced necrotic local lesions on inoculated primary leaves but no virus was recovered from the trifoliate leaves.

Among the cowpea cultivars tested, four types of host response were obtained. Brown Crowder, Brown Sugar Crowder, Blue Goose, Miss Silve, Cream Crowder, Running Acre, Blackeye Crowder White, and Tardy were immune; necrotic local lesions were obtained in Georgia 21, Long Pod Purple Hull, Tennessee White Crowder, Dixie Cream, and White Sugar Crowder; local necrotic lesions followed by systemic necrosis was observed in Burch Purple Hull, Six Weeks, and Browneye; and local chlorotic spots followed by systemic mosaic was obtained in Big Bay, Cabbage, Texas Cream 40, Keenckle Hull,

Purple Hull 49, Purple Hull Pinkeye, Purple Hull White Crowder, White Browneye Crowder, White Hull Conch, and White Acre. Brantley and Kuhn (5) observed a similar range of responses by cowpea varieties to the cowpea strain of SBMV.

Seed transmission.—Infectious virus was recovered from all floral parts tested as well as from embryos and seed coats of immature and maturing seeds. Infectivity could also be recovered from seed coats, but not from embryos of dry mature seeds. In three experiments, each involving 25 mature dry seeds, infectivity was detected from 48% (range 40-52%) of the seed coats; no infectivity was recovered from the seed coats of the same number of healthy seeds.

In seed transmission tests using mature seed of GH-infected Early Ramshorn cowpea stored for several months, only one seedling of 77 seedlings (1.3%) was infected with GH as determined by symptom expression and serological assay of all seedlings. No virus was detected in 25 seedlings from seed of virus-free cowpea. Germination in both seed lots was 100%.

Physicochemical properties.—Properties of the virus in sap were: a dilution end point of 10^{-8} (frequently infective at 10^{-7}, but not at 10^{-8}) and a thermal inactivation point (10 min exposure) at 90 C, but not at 85 C.

A value of 0.62 was obtained for the ratio of the sedimentation depth of GH to that of TMV; from this value, the sedimentation coefficient was estimated as 115 S. Analytical gradient centrifugation of SBMV, GH, and a mixture of the two, revealed only a single sedimenting peak at the same position in each gradient column, indicating that the sedimentation coefficient of GH was identical to that of SBMV. The sedimentation coefficient of SBMV is reported as 115 S (23).

Electrophoretic mobilities of -3.3×10^{-5} and -3.7×10^{-5} $cm^2\ sec^{-1}\ volt^{-1}$ were calculated for GH and SBMV in a sucrose gradient buffered at pH 7.0 with 0.01 M Na_2HPO_4 and citric acid. Hartman and Lauffer (10) obtained a mobility of -4.0×10^{-5} $cm^2\ sec^{-1}\ volt^{-1}$ for SBMV in phosphate buffer (pH 7.0) with an ionic strength of 0.02. The relationship between pH and electrophoretic mobilities of GH and SBMV in citric acid-phosphate buffer is shown in Fig. 1. Isoelectric points of 5.7 and 5.9 were obtained for SBMV and GH, respectively. Tremaine and Wright (26) found the isoelectric points of SBMV and SBMV-CS to be 5.9 and 6.0 respectively; Magdoff-Fairchild (16) reported isoelectric points ranging from pH 3.9 to 6.0 for different variants of SBMV. Fig. 1 shows that GH is more electronegative than SBMV above pH 7.1, and more electropositive below pH 7.1. Tremaine and Wright (26) found that SBMV-CS relative to SBMV is more electronegative above pH 6.2 and more electropositive below pH 6.2.

Serological studies showed that GH was antigenically related to SBMV, but that it was not identical. Antiserum to GH gave precipitin end points of 1:128 and 1:64 with homologous and heterologous antigens, respectively. With SBMV antiserum, precipitin end points of 1:64 and 1:32 were obtained. These values correspond with those reported by Shepherd and Fulton for SBMV-CS and SBMV (24).

Strain differences between GH and SBMV were also detected by gel-diffusion tests. Precipitation arcs were obtained between each virus and homologous or heterologous antiserum. Reactions of partial identity with spurs on appropriate sides of the precipitin arc were observed when the two virus isolates in adjacent wells diffused against a common antiserum. In intragel cross absorption tests, precipitin arcs formed only between homologous serum, absorbed with heterologous antigen, and homologous antigen; these results indicate that part of the antigenic determinant of GH is distinct from that of SBMV. Additional gel-diffusion experiments using GH, SBMV, and SvBMV and their respective sera, demonstrated that GH is serologically distinct from the other strains. A detailed comparison of GH with several strains of SBMV will be reported elsewhere. In immunoelectrophoresis at pH 7.5, GH and SBMV moved anodically, but GH moved faster. In an in vitro mixture of SBMV and GH, a spur was formed, the position of which corresponded to the position of the precipitin line obtained when SBMV alone was electrophoresced (Fig. 2).

Isometric particles 30 nm in diam were observed in negatively stained virus preparations; the diam is similar to that reported for SBMV in negative stain (23).

DISCUSSION.—The physical properties and serological reactions indicate that the virus is a strain of SBMV. A few major differences distinguish it from any of the known strains of SBMV. Except for SvBMV (29) none of the SBMV strains has been reported to infect reciprocal hosts (24). On the other hand, GH infected both cowpea and bean. Unlike SvBMV, it did not produce systemic symptoms in any of the bean varieties inoculated.

The results obtained in the present investigation on seed transmission indicate that GH is not transmitted through the embryo in seeds sufficiently mature for storage. GH was, however, found to be associated with the seed coat at all stages tested. Cheo (7) suggested that seedling infection of bean by SBMV was probably due to mechanical inoculation of the developing seedling with virus from infected seed coats. Cowpea being epigeal, the seed coats remain attached to the primary leaves for some time after germination of the seed. Roberts and Price (20) observed that SBMV can infect uninjured bean leaves. Based on this information, it can be inferred that the seed coat serves as the source of inoculum for the seedling or the germinating embryo, as has been proposed for seed transmission of SBMV in bean (7, 18).

Tremaine and Wright (26) observed in electrophoresis studies that SBMV-CS was more electronegative than SBMV above pH 6.2 and more electropositive below pH 6.2. The results reported here indicated that GH was more electronegative than SBMV above pH 7.15 and more electropositive below pH 7.15, indicating differences in surface charge between GH and SBMV (Fig. 2), and also that GH differs from SBMV-CS (26) in electrophoretic mobility. Grogan and Kimble (9) reported that both the severe bean strain and bean strain of SBMV moved faster than SBMV-CS in immunoelectrophoresis at pH 8.6. In the present study, SBMV appeared to move slower than GH.

The differences between GH, SvBMV, and the type

strain of SBMV indicate that GH is different from any of the known strains of SBMV. However, the differences in host range and electrophoretic mobilities between strains of a virus are not unusual since similar observations have been made for strains of TMV (25).

The properties of the GH strain suggest an evolutionary trend in the group of SBMV strains. Considering the group as a whole, we speculate that the various types possibly arose by mutation from GH or another closely related strain. Two factors could have induced the mutation. These are temp and continuous passages through a single host. The effects of high temp on plant viruses have been well documented (11, 12, 15). The various strains of SBMV are either tropical or subtropical, hence they are adapted to replicating at high temp. High temp appears to increase mutation. Mutation arising from continuous passage of virus through a single host has also been reported (17). Similarly, continuous passage of GH through bean may give rise to mutants that would infect only bean but not cowpea. Assuming that GH is the wild type of SBMV, continuous propagation of this virus through a single variety of bean or cowpea, coupled with the high temp conditions under which these crops are grown in the tropics and subtropics, could result in production of mutant strains specific to each crop.

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