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# OCCURRENCE OF SOUTHERN BEAN MOSAIC VIRUS (SBMV) IN TOGO AND ITS INTERACTION WITH SOME COWPEA CULTIVARS

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## ABSTRACT

**Cowpea (*Vigna unguiculata* L. Walp) is one of the major grain legumes in tropical Africa. This leguminous plant is susceptible to many diseases including those caused by viruses. This article reports results from surveys conducted from the main cowpea producing areas in Togo for the occurrence of Southern Bean Mosaic Virus (SBMV) and search for cowpea cultivars that are resistant to this virus. Serological tests (double diffusion in agarose gel and DAS-ELISA) with polyclonal antiserum to intact SBMV were used for the identification of the SBMV isolates. Results indicated that SBMV is widespread in the main cowpea producing areas in Togo. *Cassia hirsuta*, naturally infected by SBMV, may constitute a carryover host for SBMV between two cowpea growing seasons. Two SBMV isolates (18-10 and 10-19) cloned by local lesions transfers on susceptible cowpea were used for the screening of 58 cowpea cultivars. Cowpea cultivars IT82D-703, IT82D-786, IT83S-818, TVx1193-9F and TVx1850-01E were resistant to both isolates. These resistant cowpea cultivars could be recommended for farmers where SBMV is a serious problem for cowpea production.**

*Key Words*: Serological tests, viruses, *Vigna unguiculata*

## RÉSUMÉ

**Le niébé (*Vigna unguiculata* L. Walp) est une légumineuse à graines cultivée dans les régions tropicales d'Afrique. Cette légumineuse est très sensible à un grand nombre de maladies dont celles causées par des virus. Ce papier rapporte les résultats de prospections phytosanitaires dans les principales régions productrices de cette légumineuse au Togo et portant sur la présence du SBMV et aussi sur la recherche de cultivars de niébé résistants à ce virus. Des tests sérologiques (immunodiffusion dans l'agarose et le test DAS-ELISA) utilisant l'antiserum polyclonal à l'égard du SBMV ont permis d'identifier les isolats de ce virus. Les résultats indiquent que le SBMV est très répandu dans les principales régions productrices du niébé au Togo. *Le Cassia hirsuta* a été trouvé infecté naturellement par le SBMV, il peut servir de réservoir de virus entre deux saisons de culture du niébé. Deux isolats du SBMV, (18-10, 10-19) clonés par transfert de lésions locales sur du niébé sensible ont été utilisés pour cribler par test 58 cultivars de niébé. Les cultivars de niébé suivants : IT82D-703, IT82D-786, IT83S-818, TVx1850-01E se sont révélés résistants à l'égard des deux isolats du SBMV, et ceux-ci peuvent être recommandés aux producteurs surtout dans les régions où le SBMV est une contrainte majeure pour la production du niébé.**

*Mots Clés*: Tests sérologique, Virus, *vigna unguiculata*

## INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is cultivated in most countries in West Africa, especially in the Guinea and Sudan Savanna regions.

The average yield from farmers' fields is very low (between 200 and 300 kg $ha^{-1}$). Susceptibility to diseases and insect pests, and several other constraints, are responsible for such low yields (Singh and Allen, 1979). Many viruses have been reported as infecting cowpea in West Africa : Cowpea (yellow) mosaic virus (CPMV), Cowpea Aphid-borne Mosaic Virus (CAMV), Blackeye Cowpea Mosaic Virus (BlCMV), Cowpea Mottle Virus (CMeV), Cucumber Mosaic Virus (CMV), Cowpea Golden Mosaic Virus (CGMV), Cowpea Mild Mottle Virus (CMMV), Southern Bean Mosaic Virus Cowpea Strain (SBMV-CS) and Tobacco Mosaic Virus Cowpea Strain (TMV-CS) (Thottappilly and Rossel, 1985, 1992).

Most of these viruses often occurred in mixed infections and had detrimental effects on cowpea yields Rossel and Thottappilly, 1985; Thottappilly and Rossel, 1985, 1992; Gumedzoe *et al.*, 1990). Southern Bean Mosaic Virus (SBMV) has been reported in West Africa (Lamptey and Hamilton, 1974; Shoyinka *et al.*, 1979; Givord, 1981; Fauquet and Thouvenel, 1987; Gaikwad and Thottappilly, 1988; Gumedzoe *et al.*, 1990). In preliminary studies, SBMV was identified in Togo (Gliem, 1984; Gumedzoe *et al.*, 1990) but it was not well characterised. As an adequate knowledge of SBMV and its isolates or strains occurring in the main cowpea growing areas in Togo is a prerequisite for effective control measures to be developped against this virus, it was necessary to describe SBMV isolates identified in Togo and to search for cowpea cultivars resistant to SBMV.

This article reports results from surveys in the main cowpea production areas in Togo for the prevalence of SBMV and the characterisation of the isolates of the virus. Cowpea cultivars were screened for resistance to SBMV.

## MATERIALS AND METHODS

**Antisera and plant materials.** Polyclonal antiserum to intact SBMV (1 mg/ml) was obtained from the Biotechnology Unit at the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria). All cowpea lines were grown and maintained in insect-proof cages.

**Surveys.** Both farmers' fields and experimental plots in major cowpea production areas in Togo were surveyed for cowpea plants showing mosaic or mottling symptoms of the virus. One trifoliate leaf was collected from each of the virus-infected plants and put in separate plastic bags. The plastic bags were labelled, stored in a cool box for one or two days prior to their transfer to the laboratory where they were frozen.

**Identification of SBMV isolates.** Serological tests (double diffusion in agarose gels : 0.7 % agarose, 1 % NaCl, 0.1 % sodium azidin) 0.01 M potassium phosphate buffer at pH 7.5) were used for the identification of SBMV isolates as described elsewhere (Gumedzoe *et al.*, 1990). The gels were incubated in a moist chamber at room temperature for 24hr. After the completion of the test, the gels were photographed. In order to better visualise the precipitin lines, a simple procedure of staining these agarose gels was used as described elsewhere ( Crowle and Cline, 1977; Asselin *et al.*, 1980; Mink, 1988). In this case, the gels consisted of 1.5 % agarose, 1 % NaCl, 0.1 % sodium azide and 0.01 M potassium phosphate buffer of pH 7.5. A double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) was also used for the identification of SBMV isolates (Clark and Adams, 1977). Precoated plates from the Biotechnology Unit of the International Institute of Tropical Agriculture were used for this assay.

**Reactions of some cowpea cultivars to SBMV isolates.** Fifty eight cowpea cultivars were screened using local-lesion selected SBMV isolates 10-19 and/or 18-10. Inoculum was prepared by grinding leaves of infected cowpea cultivars in a cold sterilized mortar with a few drops of 0.01 M potassium phosphate buffer (pH7.5). Inoculations were carried out by rubbing primary leaves of one week-old cowpea seedlings dusted with 600 mesh silicon dioxide (carborandum) with the extract prepared in the phosphate buffer. Healthy seedlings inoculated

with buffer served as controls. Inoculated leaves were immediately washed with tap water. At least five cowpea seedlings (including the control) were inoculated at each time. Inoculated plants were scored for infection between seven and thirty days after inoculation by counting the number of plants showing symptoms on inoculated primary leaves and younger trifoliate leaves (mottle, mosaic).

The scale used to rate the symptom of SBMV on cowpea cultivars inoculated with this virus was as follows.

Score 1: No symptom in plants (no visible mottle or mosaic).
Score 2: Slight mosaic symptom (1 to 25% of the cowpea cultivars inoculated were infected).
Score 3: Moderate mosaic symptom (25 to 50 % of plants infected).
Score 4: Severe symptoms with stunting (50 to 75 % of plants infected).
Score 5: Highly severe symptoms with stunting (75 to 100% of plants infected).

## RESULTS

**Surveys, identification and prevalence of SBMV in various cowpea producing areas in Togo.** The aim of this study was to establish the distribution and incidence of SBMV in various cowpea producing areas in Togo. From the 705 field samples examined, 63.12 % reacted positively to at least one of the antiserum of the seven major cowpea viruses currently studied in our laboratory (BICMV, CAMV, CMeV, CMMV, CMV, CPMV and SBMV). The incidence of SBMV in the samples assayed was 13.86 % on cowpeas and non-cultivated plants from the following prefectures : Bassar, Golfe, Haho, Kloto, Kozah, Ogou, Oti, Sotouboua, Tone, Vo, Yoto and Zio (Fig. 1). Virus mixtures were identified in single plants and ranged from three viruses to two of the cowpea viruses in various combinations (i.e. SBMV + CPMV + CAMV and SBMV + CPMV). These mixed infections were recorded using serological tests (DAS-ELISA and double diffusion in agarose gels) and they included two or more cowpea viruses. Various non-cultivated plants, including *Cassia hirsuta*, were also found infected with SBMV. The SBMV isolate 10-19 was recovered from cowpea cultivar IT86D-901 in experimental plots of the "Societe Togolaise du Coton" (SOTOCO) at the locality Adza Yao in Ogou prefecture.

SBMV isolate 18-10 was recovered on cowpea at Vogan in Vo prefecture. These two viral isolates were cloned by local lesion transfers on the cowpea cultivar IT81D-985 before being used to screen the cowpea cultivars.

In the immune double diffusion assay, precipitin lines normally appeared about 16 to 24 hr after leaf extracts and the SBMV antiserum were added to wells (Fig. 2). Precipitin lines stained with Crocein scarlet in combination with Coomassie blue were quite distinct and remained visible after long storage. The immunoprecipitin lines stained with the combination of Crocein scarlet 7B and Coomassie brillant blue were more distinct than those stained with Coomassie blue alone. There

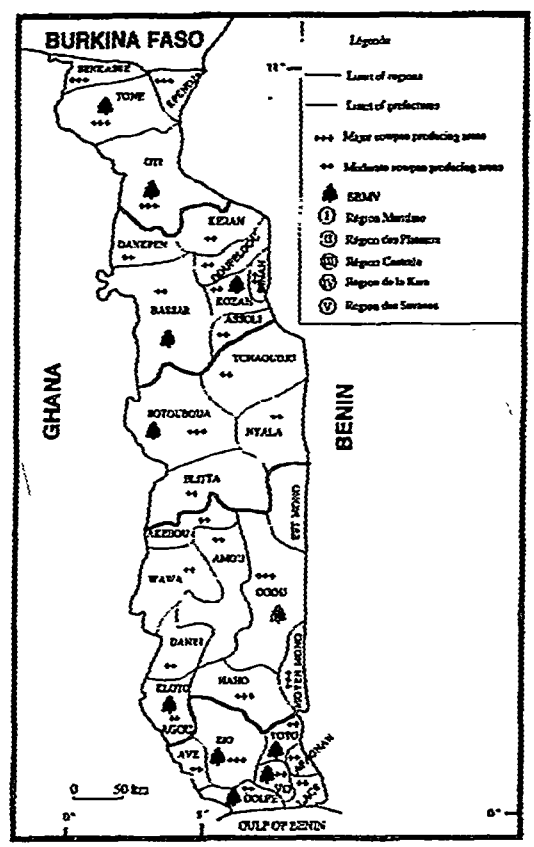


Figure 1. The geographical locations of the prefectures surveyed so far in Togo, and where SBMV was detected.

were some black smears around the reactant wells with SBMV isolates.

**Reactions of cowpea cultivars and other plant species to SBMV isolates 10-19 and 18-10.** Cowpea cultivars were categorised as highly susceptible (score 4 to 5), moderately susceptible (score 2 to 3) and resistant (score 1), according to the severity and the type of symptoms induced by the two cloned SBMV isolates (10-19 and 18-10). Most of the cowpea cultivars tested using both SBMV isolates were highly to moderately susceptible. Resistance of cowpea cultivars to both virus isolates was proved if in both tests, infectivity to a susceptible cowpea and immunodiffusion in agarose gels, were negative. The following cowpea cultivars were resistant to both SBMV isolates : IT82D-703, IT82D-786, IT83S-818, TVx1850-01E (Tables 1 and 2).

Symptoms observed on inoculated leaves of most cowpea cultivars included the development of necrotic or chlorotic local lesions. Systemic reactions included varying degrees of mosaic leaf distortion and plant stunting. Severity of symptoms varied with SBMV isolates infecting the same cowpea cultivar. For example, the cowpea cultivar Locale Blanche is susceptible to isolate 10-19 but was found resistant to isolate 18-10. Likewise, isolate 10-19 readily infected the cowpea cultivar IT81D-985, whereas isolate 18-10 did not easily infect this cultivar. SBMV isolate 10-19 failed to infect the following plant species : *Cajanus cajan*, *Phaseolus vulgaris* cv. Saint-Fiacre and Saxa, *Chenopodium amaranticolor*, *Crotalaria* sp. and *Glycine max* cv Jupiter (Data not shown). One species of *Mucuna* reacted with local lesions when inoculated with SBMV isolate 10-19.

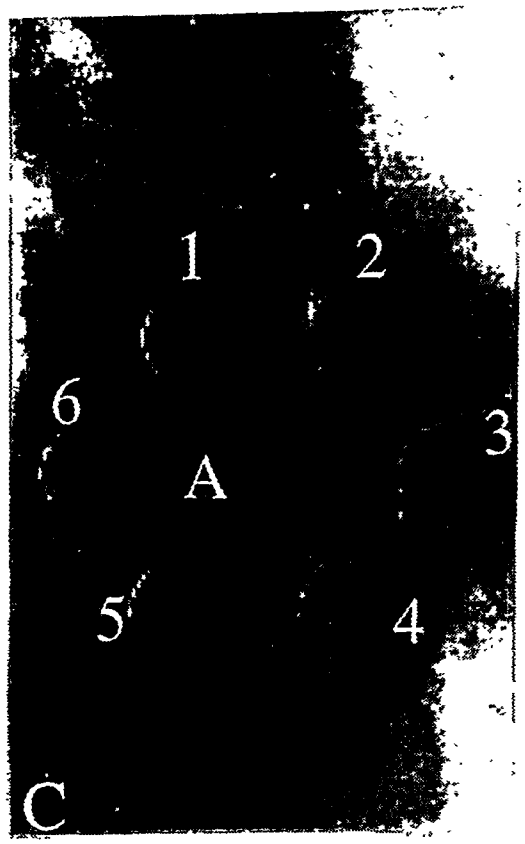


Figure 2. Immuno-diffusion tests in agarose gel (0,7 % agarose, 1% NaCl, 0.1% $NaN0_3$ in Phosphate buffer 0.01 M pH 7.5).
Center and peripheral wells were filled with polyclonal antiserum to intact SBMV (A) and diseased and healthy plant extracts from the cowpea cultivar IT82D-703.
1 extract from non-infected plant cultivar IT82D-786.
2,4 and 5 were infected plant extracts from cowpea cultivars IT85F-2085, IT84S-2246-4 and IT81D-1007 respectively.

## DISCUSSION

Among isometric viruses infecting cowpea, SBMV is one of the more important. It has been identified in Togo and in other areas of Africa (Lamptey and Hamilton, 1977; Shoyinka *et al.*, 1979; Givord, 1981; Thottappilly and Rossel, 1985; Fauquet and Thouvenel, 1987). Results from the present study confirmed this wide distribution of SBMV throughout the cowpea producing areas in Togo. Similar observations were made earlier in this country but no research was conducted to search for cowpea cultivars that are resistant to this virus (Gliem, 1984; Gumedzoe *et al.*, 1990). Most of the cowpea cultivars that are popular in major cowpea producing areas in Togo (58-146, IT81D-985 or VITOCO and VITA5) were highly to moderately susceptible to the SBMV isolates studied. A number of cowpea cultivars (IT82E-16, IT82D-889, IT83S-818, TVu19) were resistant to these viral isolates as previously reported (Ladipo and Allen, 1979; Singh and Ntare, 1985; Singh *et al.*, 1992). The use of these resistant cowpea cultivars could help reduce considerably the losses caused by this

TABLE 1. Reactions of 36 cowpea cultivars to SBMV isolate 18-10

| Cowpea cultivars | Symptoms | Ratio of infected plants to number of inoculated plants | Score | Immuno-diffusion test |
|---|---|---|---|---|
| 1) IT81D-1007 * | Mo, df | 4/4 | 3 | (+) |
| 2) IT81D-1137 | MT, df | 4/4 | 5 | (+) |
| 3) IT81D-889 | mild Mo | 3/4 | 5 | (+) |
| 4) IT82D-889 | mild Mo | 4/4 | 3 | (+) |
| 5) IT82D-786* | - | 0/4 | 1 | (-) |
| 6) IT82D-703* | - | 0/4 | 1 | (-) |
| 7) IT82D-885 | - | 0/4 | 1 | (-) |
| 8) IT82E-18 | Mt,df,STN | 4/4 | 4 | (+) |
| 9) IT82E-60 | - | 0/4 | 1 | (-) |
| 10) IT82E-9* | mild,Mo | 4/4 | 3 | (+) |
| 11) IT82E-16* | - | 0/4 | 1 | (-) |
| 12) IT82E-32 | Mt,df | 3/4 | 4 | (+) |
| 13) IT83S-818* | - | 0/4 | 1 | (-) |
| 14) IT84S-2246-4 | Mt,df | 4/4 | 5 | (+) |
| 15) IT84E-124 | - | 0/4 | 1 | (-) |
| 16) IT84E-108 | - | 0/4 | 1 | (-) |
| 17) IT85D-3516-2 | Mo,df | 4/4 | 5 | (+) |
| 18) IT85F-2085 | Mo,df | 4/4 | 3 | (+) |
| 19) IT85F-2805 | Mo,df | 4/4 | 5 | (+) |
| 20) TVu19 | - | 0/4 | 1 | (-) |
| 21) TVu410 | | 0/4 | 1 | (-) |
| 22) TVu645 | | 0/4 | 1 | (-) |
| 23) TVx1193-9F* | - | 4/4 | 4 | (+) |
| 24) TVX3236-01G* | Mo | 4/4 | 4 | (+) |
| 25) TVx3671-14C-01D | Mo | 2/4 | 2 | (+) |
| 26) TVx1850-01E* | - | 0/4 | 1 | (-) |
| 27) 58-146* | mild Mo | 4/4 | 3 | (+) |
| 28) Ife Brown* | mild Mo | 4/4 | 3 | (+) |
| 29) Diapaga | - | 0/4 | 1 | (-) |
| 30) KN1 | Mo,df,STN | 4/4 | 5 | (+) |
| 31) Locale blanche* | - | 0/4 | 1 | (-) |
| 32) Locale Glél* | Mo | 4/4 | 3 | (+) |
| 33) Locale Afagnangan | - | 0/4 | 1 | (-) |
| 34) IT81D-985* (VITOCO) | Mo | 4/4 | 3 | (+) |
| 35) VITA7 | Mo | 2/4 | 2 | (+) |
| 36) IT86D-1056 | Mo | 3/4 | 3 | (+) |

Mo: Mosaic
df: leaf deformation
Mt: mottling
STN: Stunting of infected plants
*: Cowpea cultivars inoculated with both SBMV isolates
(+) Positive reactions
(-) negative reactions

virus in cowpea growing areas where SBMV is a common problem (O' Hair *et al.*, 1981; Collins *et al.*, 1985). Various non-cultivated plants, including *Cassia hirsuta* were found infected with SBMV. This finding may have some epidemiological implications if the two leguminous plants (cowpea and *Cassia hirsuta*) occur in the same field. The non-cultivated *Cassia hirsuta* may constitute a carryover host for SBMV between two cowpea growing seasons in some areas.

The effective management of plant virus diseases requires the use of rapid, reliable and sensitive methods of detection (Hamilton, 1992). In Africa generally, laboratory facilities are inadequate for sophisticated studies and characterisation of viral genomes (like the use of cDNA, molecular hybridisation etc.). However,

TABLE 2. Reactions of cowpea cultivars to SBMV isolate 10-19

| Cowpea cultivars | Symptoms | Systemic Reactions | Score | Immuno-diffusion test |
|---|---|---|---|---|
| 1) IT81D-985* (VITOCO) | NLL,CLL | VC,Mo,STN | 4 | (+) |
| 2) IT81D-1007 * | NLL | VC,Mo | 4 | (+) |
| 3) IT82D-703* | - | - | 1 | (-) |
| 4) IT82D-786* | - | - | 1 | (-) |
| 5) IT82D-812 | NLL | Mo | 3 | (+) |
| 6) IT82D-885 | - | GMo | 2 | (+) |
| 7) IT82E-9* | - | Mo | 3 | (+) |
| 8) IT82E-16* | NLL | VC,Mo | 3 | (+) |
| 9) IT83S-818* | - | - | 1 | (-) |
| 10) IT83S-962 | NLL | VC,Mo | 2 | (+) |
| 11) IT2945-01D | NLL | GMo | 4 | (+) |
| 12) TVx1193-9F* | - | - | 1 | (-) |
| 13) TVx1850-01E* | - | - | 1 | (-) |
| 14) TVx3236-01G* | - | - | 1 | (-) |
| 15) TVx3671-7C-02D | NLL | VC,Mo | 2 | (+) |
| 16) TVx3671-14C-01D | - | GMo | 2 | (+) |
| 17) VITA5 | - | Mo | 3 | (+) |
| 18) 58-146* | - | Mo | 1 | (+) |
| 19) Locale blanche* | - | Mo | 4 | (+) |
| 20) Locale Niamtougou | NLL,CLL | Mo,Vb | 5 | (+) |
| 21) Locale Tsededzi | NLL | Mo,STN | 5 | (+) |
| 22) Locale Glél* | - | Mo | 5 | (+) |
| 23) Ife Brown* | - | Mo | 5 | (+) |

CLL : Chlorotic local lesions
NLL : Necrotic local lesions
Mo : mosaic
VC : Vein-clearing
Vb : Vein-banding
STN : Stunting
* : Cowpea cultivars inoculated with both SBMV isolates
(+) : Positive reactions
(-) : Negative reaction

cowpea viruses can be identified using simple serological methods like agar gel diffusion tests (Thottappilly and Rossel, 1992). Serological tests especially immunodiffusion tests in agarose gels using polyclonal antisera, have been widely applied for many years for the rapid detection and identification of plant isometric viruses like SBMV.

This serological test, however, has the following disadvantages: it uses much antiserum and is not sensitive, but modifications have been devised to overcome these difficulties. The immune double diffusion assay also cannot be used with sap from natural hosts because of low virus concentration, or presence of factors (such as mucilages, tannins etc.) in sap which interferes with the reaction. Moreover, precipitation lines disappear quickly in few days and it is necessary to photograph the gel before the precipitation lines disappear.

To overcome these problems, especially the preservation of precipitation lines, some improvements like staining the precipitation lines were developed by different workers (Crowle and Cline, 1977; Mink, 1988). We applied successfully the staining technique of Mink (1988) to the SBMV isolate 18-10.

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