Acta Bot. Croat. *46*, 15—21, 1987.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 576.858.8:582.738(497.1)=20

IDENTIFICATION AND CLASSIFICATION OF SOYBEAN MOSAIC VIRUS ISOLATES FOUND IN KOSOVO (YUGOSLAVIA)

NYSRET TARAKU[1], *SUE A. TOLIN*[2] and *NIKOLA JURETIĆ*[3]

([1]Institute for Plant Production, Peć, Faculty of Agriculture, University of Priština, [2]Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, and [3]Department of Botany, Faculty of Science, University of Zagreb)

Received January 20, 1987

Three virus isolates of soybean mosaic virus (SBMV) have been isolated from soybean cultivated in the Province of Kosovo (southeastern part of Yugoslavia). It has been established on the basis of test-plant reactions, serology, virus stability in sap and type of virus particle. The isolates are identical with each other and belong to Gl-strain group of SBMV. It has been found by ELISA-test that the isolates studied occur in the same concentration in endosperm and embryo of soybean seed produced in infected plants.

Introduction

In 1984 three virus isolates were found on soybean in the Province of Kosovo in Yugoslavia: two isolates originated from plants growing in fields and one from commercial seed lots. Preliminary investigations indicated that these isolates could belong to soybean mosaic virus (SBMV).

SBMV occurs in soybean all over the world. It was found in Yugoslavia as well. Spasić (1961) and Nikolić and Stakić (1964) have isolated SBMV in the northeastern part of Yugoslavia. However, so far this virus has not been isolated in southeastern regions of Yugoslavia. Generally, the SBMV isolates described up to now were usually similar to the type strain in host range and symptomatology (Bos 1972), although some isolates may have exhibited distinctively different pro-

perties belonging to strains described by Ross (1969) or by Cho and Goodman (1979). SBMV is a member of the potyvirus group (Mattews 1979, Edwardson 1974). Its properties have been summarized by Bos (1972) and Edwardson and Christie (1986).

This paper deals with three SBMV isolates including their host range, symptomatology, light microscope inclusion bodies and particle size and morphology. Additionally, three Yugoslav isolates were compared on the basis of reactions of soybean differential cultivars with seven SBMV isolates characterized previously.

Material and Methods

All the three isolates were found in the Province of Kosovo (southeastern part of Yugoslavia). Two isolates designated SK and SV were isolated in 1984 from soybean (not determined cultivar) plants growing in commercial fields in Klina and Vitina Counties respectively, and the third isolate (SP) was isolated from a commercial seed lot. All isolates were mechanically transmitted and maintained in the greenhouse in Essex or Lee soybean. Inocula were prepared by homogenizing leaves or homogenizing soybean seed with chilled mortar and pestle in 0.01 M sodium phosphate buffer pH 7. The inocula were mechanically inoculated by Carborundum onto primary leaves.

In host range tests species listed in Table 1 were used. All three isolates were also inoculated onto soybean differential cultivars which included some SBMV-susceptible and some SBMV-resistant cultivars. To detect latent virus infection in test plants, the sap of those plants was back inoculated to Essex soybean. Our isolates SK, SV and SP were compared with eight isolates of SBMV: G1-Va, G1-IL, G4-IL, G5-IL, G1-5-IL, G6-IL, and G7-IL which were characterized symptomatologically by Ross (1969), Cho and Goodman (1979) and Hunst and Tolin (1982).

The properties in vitro were determined in the standard manner. Serological experiments in agar-gel double diffusion tests were performed by crude sap extracts in 0.6% agar, containing 0.2% sodium dodecyl sulphate, 0.1% sodium azide and 0.6% sodium chloride. Antiserum to type strain of SBMV (titer 1 : 64) from the collection of the second author was used.

The seed of infected plants with spotting symptoms were tested by ELISA technique: the seed were put in distilled water and after 24 hr the endosperm was separated from the embryo. Later on endosperm and embryo were homogenized separately and filterd through Watmann No. 1 filter paper. The filtrates were then tested by ELISA. Antiserum against SBMV included in this test was the same as quoted above. SBMV gama-globulin was used at 1 or 2 mg/ml, and alkaline phosphatase-conjugated globulin was employed in dilutions 1 : 1000, 1 : 1500 and 1 : 2000. Incubation times for gama-globulin samples, conjugate and substrate were 1 and 2 hr at 30°C. Seed tested samples were prepared either by 20 endosperms or 20 embryos.

Results and Discussion

1. *Host range and symptoms*

Results of the host range studies are shown in Table 1. Our isolates SK, SV and SP produced a mild mosaic and veinclearing on soybean Essex and Lee and spotting on seed (Fig. 1 C). By contrast all three isolates produced — on inoculated leaves of soybean Ogden and Marshall — local necrotic symptoms only. However, these three isolates could not infect soybean York and Kwanggyo. The investigated isolates caused similar symptoms on other species as well. Our isolates produced necrotic lesions on bean (*Phaseolus vulgaris* L. cv. Topcrop) and on cowpea (*Vigna unguiculata* L. cv. Walp Blackeye), and systemic and mild mottle on *Pisum sativum* only. The three isolates could not infect clovers *T. pratense, T. repens* and *T. incarnatum.* In general, the host range and test plant reactions of isolates SK, SV and SP markedly resembled those of SBMV. Since all three ioslates caused identical reactions on the test plants used, only isolate SP, as a representative, was included in further symptomatological comparisons of our isolates with some defined SBMV isolates.

Isolate SP and the 7 defined SBMV isolates were parallelly inoculated onto soybean differential cultivars. Their reactions are shown in Table 2. As can be seen from the Table, SP isolate could not be distinguished on the basis of these reactions from SBMV isolates G1-Va and G1-IL. However, it could be distinguished from other SBMV isolates. Therefore, it seems that by its symptomatological properties our SP isolate can be classified in the G1-strain group (comp. *Cho* and *Goodman* 1979).

Table 1. Host range study of three soybean mosaic virus isolates

Host	Symptoms caused by virus isolates*		
	SP	SK	SV
Glycine max cv. Essex	MMo, Vc (Fig. 1B)	MMo, Vc	MMo, Vc
G. max cv. Lee	MMo, Vc	MMo, Vc	MMo, Vc
G. max cv. non det.	MMo, Vc (Fig. 1A)	MMo, Vc	MMo, Vc
G. max cv. Ogden	Ln	Ln	Ln
G. max cv. Marshall	Ln	Ln	Ln
G. max cv. York	Ns	Ns	Ns
G. max cv. Kwanggyo	Ns	Ns	Ns
Phaseolus vulgaris cv. Topcrop	LNLe	LNLe	LNLe
Pisum sativum	MMo	MMo	MMo
Trifolium pratense	Ns	Ns	Ns
T. repens	Ns	Ns	Ns
T. incarnatum	Ns	Ns	Ns
Vigna unguiculata cv. Blackeye	LNLe	LNLe	LNLe

* Symbols for symptoms:
Ln = local necrosis, MMo=mild mosaic, LNLe = local necrotic lesions, Ns = = no symptoms, Vr = veinclearing

2. *Stability in sap*

The following properties in vitro were established: thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV). The experiments showed that our isolates had TIP between 58 and 60 °C, DEP about 10^{-2} and LIV about 3 days at 24°C. These data also showed that the investigated isolates can belong to SBMV.

3. *Serology*

In serological tests performed in agar-gel all the three isolates reacted positively with the antiserum against the typical strain of SBMV. When the three isolates were compared simultaneously with each other, precipitin lines were completely fused without any spur formation which showed a serological relationship among them (Fig. 2A-I). Also, serological reactions suggested that our isolates are more related to isolates G1-Va and G1-IL (G1-strain group, see Fig. 2A-II) than to isolates G7-IL and G6-IL (Fig. 2A-III, IV) (comp. *Ross* 1969, *Cho* and *Goodman* 1979, *Hunst* and *Tolin* 1982). These data are in concordance with the ones obtained with test plant reactions.

ELISA test assays of endosperm and embryo of soybean seed produced in soybean plants infected with our three isolates were positive. These results showed that there is no significant difference in respect of the virus concentration in the endosperm and embryo of soybean (Table 3).

4. *Light and electron microscope investigations*

Our SBMV isolates brought about the appearance of amorphous cytoplasmatic inclusion bodies. The bodies could be seen by light microscope in leaf epidermal cels of *Glicine max* cv. Essex. The bodies were oval in shape and in contact with the nucleus. They were usually somewhat smaller than the nucleus (Fig. 2C, D). An electron microscope analysis of crude infected sap by dipping method revealed anisometric flexuous virus particles of about 750 nm length (Fig. 2B).

On the basis of all above quoted data it is unambiguous that our three isolates belong to SBMV. In addition, these isolates probably belong to the typical strain of SBMV.

Fig. 1. **A—C** Symptoms caused by SP-isolate on soybean: **A** *Glycine max* cv. non det. — left healthy leaf, right leaves with mild mosaic symptoms; **B** *Glycine max* cv. Essex: leaf with systemic veinclearing; **C** seed of *Glycine max* cv. Essex: above healthy seed, below infected seed with spots; **D** Symptoms on *Glycine max* cv. Essex caused by G7-IL isolate of SBMV; **E** Symptoms on *Glycine max* cv. Lee caused by G6-IL isolate of SBMV.

Fig 2. **A** immunodiffusion reactions in agar gel double diffusion tests obtained during serological comparison of isolates SK, SP and SV with several defined SBMV isolates (the wells were filled with the folloing virus isolates: 1 = SP, 2 = SK, 3 = SV, 4 = GL—Va, 5 = Gl—IL, 6 = healthy sap, 7 = G7—IL, 8 = G6—IL, central wells contained antiserum to typical SBMV, for details see text); **B** virus particles of SP isolate in leaf dip preparation of infected *Glycine max* cv. Essex, bar represents 200 nm; **C, D** inclusion bodies (x) provoked by SP isolate in epidermal cells of *Glycine max* cv. Essex.

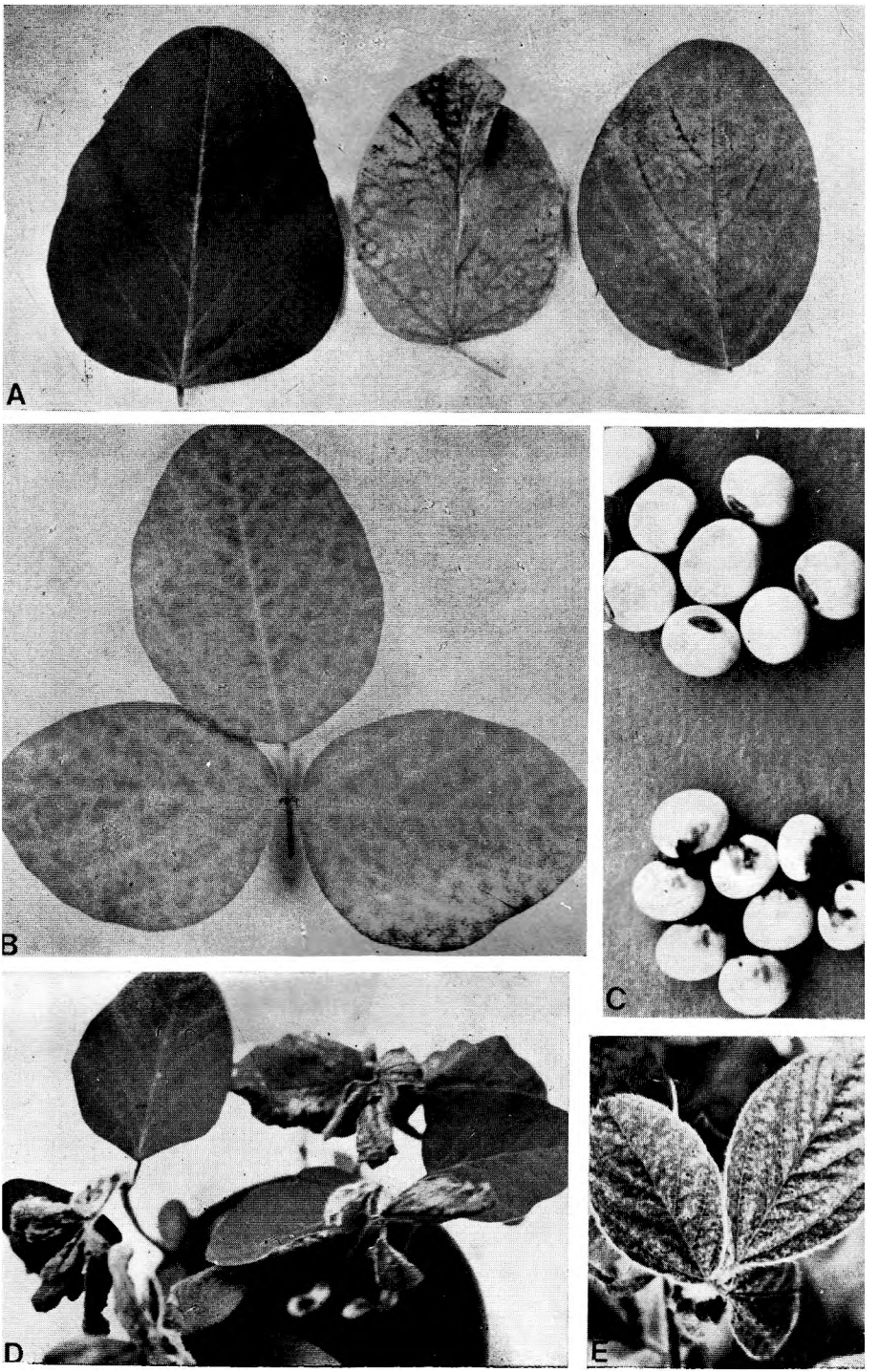

Fig. 1.

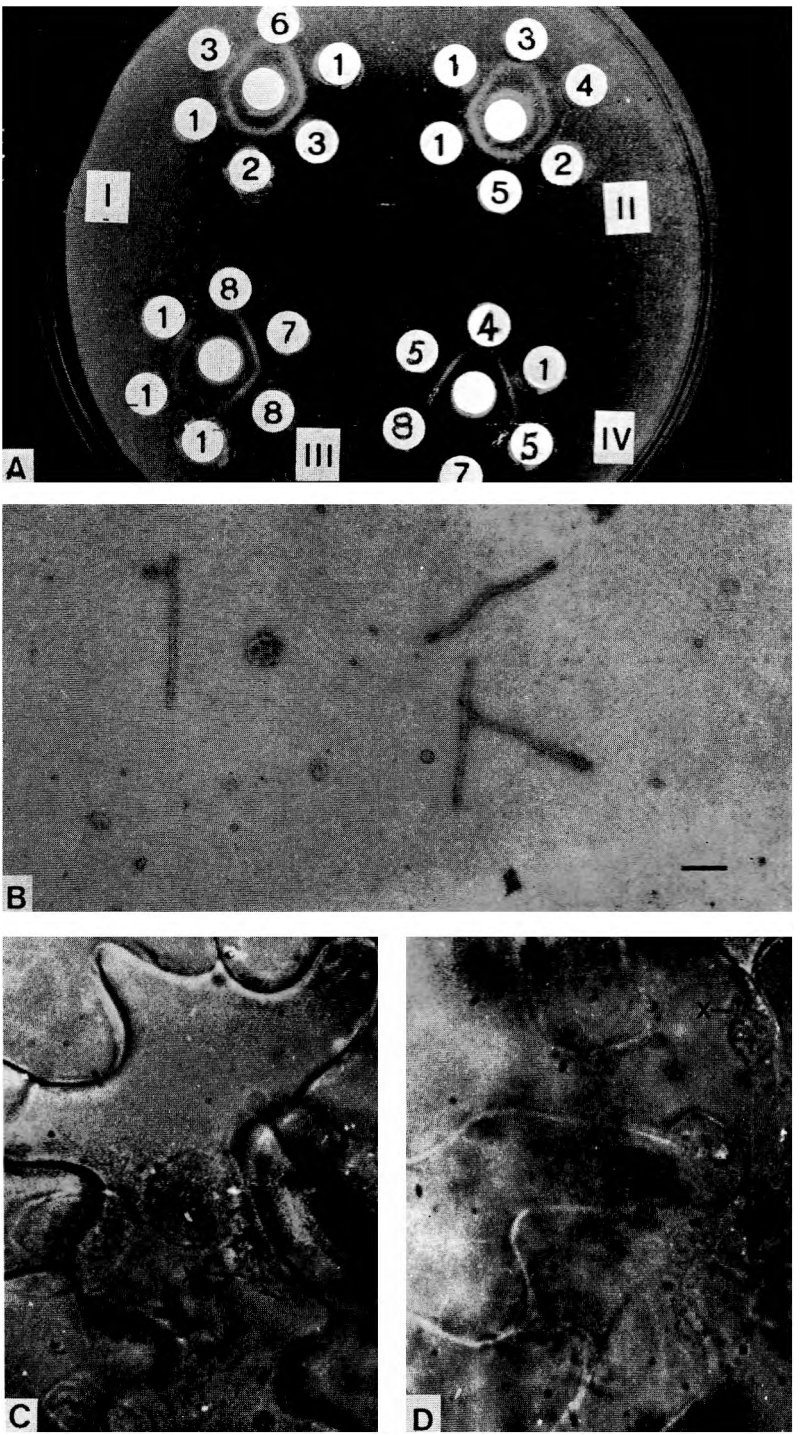

Fig. 2.

Table 2. Reactions of soybean differential cultivars to inoculations with the SP isolate studied and seven defined isolates of soybean mosaic virus (SBMV)

Soybeans	Symptoms*							
	SP	G1—Va	G1—IL	G4—IL	G5—IL	G1—5—IL	G6—IL	G7—IL
Cultivar non det.	MMo, Vc	MMo, Vc	MMo, Vc	SMo	SMo	SMo	SMo	SMo, Sn
Essex	MMo, Vc	MMo, Vc	MMo, Vc	SMo	SMo	SMo	SMo	SMo, Sn
Lee	MMo, Vc	MMo, Vc	MMo, Vc	SMo	SMo	SMo	SMo	SMo, Sn
Ogden	Ln	Ln	Ln	Ln, Vn	Ns	Ln, Vn	Ln, Vn	Ln, Vn
Marshall	Ln	Ln	Ln	Ln, Vn	Ln, Vn	Ln, Vn	Ln, Vn	Ln, Vn
Kwanggyo	Ns	Ns	Ns	Vc	Ln, Vc	Ln	Ln, Vc	Ln
York	Ns	Ns	Ns	Sn	Sn	Ln, Mo	Ln, MMo	Ln, Mo

* Symbols for symptoms:
MMo = mild mosaic, Ln = local necrosis, Ns = no symptoms, Vc = veinclearing, SMo = severe mosaic, Vn = veinnecrosis, Sn = severe necrosis, Mo = mosaic

Table 3. ELISA values for endosperm and embryo of soybean (*G. max cv. non det.*) infected seed (with spotting symptoms) tested for soybean mosaic virus (SBMV)

	Absorbance values at 405 nm			
	Endosperm		Embryo	
Reading after:	1 hr	2 hr	1 hr	2 hr
Sample No.		Infected seed		
1	0.126	0.294	0.172	0.370
2	0.224	0.486	0.245	0.529
3	0.245	0.475	0.219	0.462
4	0.247	0.535	0.239	0.499
5	0.163	0.351	0.182	0.253
6	0.159	0.373	0.174	0.431
7	0.292	0.710	0.355	0.786
8	0.411	0.779	0.466	0.863
9	0.229	0.481	0.132	0.330
10	0.190	0.426	0.203	0.478
11	0.162	0.346	0.167	0.371
12	0.229	0.514	0.166	0.379
Average:	0.223	0.480	0.226	0.479
		Virus free seed		
1	0.201	0.209	0.097	0.105
2	0.145	0.191	0.079	0.086
3	0.187	0.207	0.042	0.072
4	0.166	0.169	0.084	0.096
5	0.136	0.139	0.050	0.072
6	0.139	0.163	0.060	0.078
7	0.099	0.085	0.042	0.054
8	0.104	0.091	0.084	0.098
9	0.116	0.129	0.094	0.055
10	0.094	0.083	0.101	0.091
11	0.086	0.065	0.090	0.086
12	0.067	0.031	0.104	0.067
Average:	0.128	0.130	0.077	0.080
Control:	SBMV-infected leaf		Grounding buffer + buffered saline	
1	0.315	0.556	0.097	0.196
2	0.336	0.557	0.045	0.129
3	0.381	0.663	0.072	0.160
4	0.322	0.565	0.035	0.106
5	0.199	0.416	0.104	0.106
6	0.252	0.438	0.041	0.077
Average:	0.300	0.532	0.065	0.129

References

Cho, E., R. M. Goodman, 1979: Strains of soybean mosaic virus: Classification based on virulence in resistant soybean cultivars. Phytopathology 69, 467—470.

Bos, L., 1972: Soybean mosaic virus. No. *93* in: CMI/AAB Descriptions of Plant Viruses. Cmmonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.

Edwardson, J. R., R. G. Christie, 1968: Viruses infecting forage legumes. Vol. II. Flor, Agric. Exp. Stn. Monogr. 14, 502 pgs.

Edwardson, J. R., 1974: Some properties of the potato virus-Y group. Flor. Agric. Exp. Stn. Monogr. 4, 398 pgs.

Hunst, P. L., S. A. Tolin, 1982: Isolation and comparison of two strains of soybean mosaic virus. Phytopathology *72,* 710—713.

Matthews, R, E. F., 1979: Classification and nomenclature of viruses. Intervirology *12,* 131—296.

Nikolić, V., D. Stakić, 1964: Mozaik soje u Jugoslaviji. Suvremena poljoprivreda *12,* 683—695.

Ross, J. P., 1969: Pathogenic variation among isolates of soybean mosaic virus. Phytopathology *59,* 829—832.

Spasić, M., 1961: Prilog poznavanju parazitne flore na gajenom bilju u Timočkoj krajini. Zaštita bilja *63/64,* 32—39.

SAŽETAK

IDENTIFIKACIJA I KLASIFIKACIJA IZOLATA VIRUSA MOZAIKA SOJE NAĐENIH NA KOSOVU

Nysret Taraku[1], *Sue A. Tolin*[2] *i Nikola Juretić*[3]

([1]Institut za biljnu proizvodnju u Peći, Poljoprivredni fakultet Sveučilišta u Prištini, [2]Zavod za biljnu patologiju i fiziologiju Sveučilišta u Blacksburgu (Virginia), [3]Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Iz primjeraka soje gajene na Kosovu izdvojena su tri virusna izoalta koji pripadaju virusu mozaika soje (SBMV). Dva su izolata potjecala iz biljaka gajenih u polju (izolati SK i SV), a treći je izolat (SP) nađen u merkantilnom sjemenu soje. Da izolati pripadaju SBMV virusu, utvrđeno je na osnovi reakcija na pokusnim biljkama, seroloških osobina, njihova vladanja *in vitro* te na osnovi tipa virusnih čestica. Nađeni su izolati međusobno identični i najvjerojatnije pripadaju G1-skupini sojeva SBMV-a. S pomoću testa ELISA utvrđeno je da studirani izolati dolaze u istim koncentracijama u endospermu i embriju sjemenaka inficiranih biljaka.

Dr. Nysret Taraku
Institut za biljnu proizvodnju
YU-38300 Peć (Jugoslavija)

Prof. dr. Sue A. Tolin
Department of Plant Pathology and Physiology,
Virginia Polytechnic Institute and State University,
Blacksburg 24061, USA

Prof. dr. Nikola Juretić
Botanički zavod PMF
Maruličev trg 20/II
YU-41000 Zagreb (Jugoslavija)