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PARTIAL CHARACTERIZATION OF *SUNN – HEMP MOSAIC TOBAMOVIRUS* (SHMV) ISOLATED FROM BEAN PLANTS (*PHASEOLUS VULGARIS* L.)

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Abstract. This work presents some properties of *Sunn-hemp mosaic tobamovirus* (SHMV) orginally isolated from bean plants. Virus infected host range and induced symptoms that were typical for SHMV. Following plant species distinquished SHMV from tobacco mosaic tobamovirus (TMV): *Phaseolus vulgaris*, *Pisum sativum*, *Lupinus albus* and *Lycopersicon esculentum*. In immunoblotting the serum against SHMV did not react with TMV and *Tomato mosaic tobamovirus* (ToMV). The electrophoretical patterns of whole virions and capsid proteins were characteristic for SHMV and different from that of TMV and ToMV.

Key words: *Sunn-hemp mosaic tobamovirus*, host range, electron microscopy, serology, gel electrophoresis of virions and coat proteins

I. INTRODUCTION

In Poland, bean plants (*Phaseolus vulgaris*) is mainly infected by *Bean yellow mosaic potyvirus* and rarely by *Bean common mosaic potyvirus*. Recently, we isolated tobamovirus from plants grown from infected seeds under greenhouse condition, that on the basis of the reaction of some test plants and electron microscopy was identified as SHMV. Up to now in Poland occurred two tobamoviruses: *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV) but recently two new tobamoviruses namely, *Cucumber green mottle mosaic virus* (CGMMV) and SHMV were isolated (Pospieszny et al. 1996). We report here the partial characterization of SHMV isolated from bean. Brief report of initial studies also was published (Pospieszny et al. 1996).

II. MATERIALS AND METHODS

1. Host range and symptomatology

Plants used in this study to distinguish SHMV from TMV were: *Phaseolus vulgaris* cvs. 'Fana' and 'Pinto'; Nicotiana tabacum cv. 'Xanthi nc', *Nicotiana glutinosa*, *Pisum sativum* and *Lycopersicum esculentum*. Reaction of others plants to SHMV was also tested. Plants used for host range (recorded in Table), were grown in a greenhouse. After mechanical inoculation, the plants were maintained for at least 3 weeks to observe the appearance of the symptoms. *N. tabacum* cv. 'Xanthi nc' and *Ph. vulgaris* were used as bioassay plants for back assay of symptomless plants for SHMV and TMV, respectively.

2. Purification

SHMV was purified from infected bean plants by extraxtion with 0.1 M citric buffer pH 7.2, and chloroform clarification. Virus was concentrated by precipitation with polyethylene glycol (PEG-6000) followed by low and high-speed centrifugation. Partially purified virus was further purified by centrifugation in 10-40% sucrose density gradient. The virus band was collected from sucrose gradient and concentrated by high-speed centrifugation and resuspended in a small volume of distilleted water or buffer. TMV was purified from infected *N. tabacum* cv. Samsun. Concentration of the purified viruses was determinated spectrophotometrically.

3. Electron microscopy

Crude sap from infected leaf and purified virions were sprayed on formvar coated 300-mesh copper grids, stained with 2% phosphotungstic of acid (adjusted to pH 7.2) and examinated using electron microscope. For ultrathin section observation the tissues from infected bean leaf were fixed in 3% glutaraldehyde, postfixed in 1% osmium tetraoxide and after dehydration the material was embedded in epoxy resin Epon 812. Ultrathin section was doubly stained with uranyl acetate and lead citrate.

4. Serology

Antisera to purified viruses were prepared in the rabbit by three subcutaneous injections at 2 week intervals, with 1 mg of each virus. The antisera taken from rabbit 10 days after the last injection were used for the experiments. Serological relationships among SHMV, TMV and ToMV were evaluated against antisera to SHMV and TMV by western-blot method.

After SDS polyacrylamide gel electrophoresis (PAGE) the proteins were transferred into Immobilon-P transfer membrane Millipore (Chen et al. 1996a). Gel after electrophoresis and transfer membrane before transfer were equilibrated with transfer buffer containing: 25 mM Tris-HCl pH 7.8, 192 mM glycine, and 15% methanol, for 15 min. The transfer was conducted in Trans-blot apparatus for semi-dry transfer for 45 min. at 20 V following manufacturer protocol (Bio-Rad). After transfer completed the uncoated membrane sites were blocked with agitation at 4°C overnight in TBS-T buffer (50 mM Tris HCl pH 7.8, 150 mM NaCl, 0.05% Tween) containing 1.5% nonfat milk (Chen et al. 1996a; 1996b). The membrane was washed with TBS-T and incubated with agitation for 1 hr. at 37°C with primary antibody (2 ml in 10 ml) in TBS-T containing 1.5% milk. The membrane was washed 3-times for 10 min. each with TBS-T buffer, then incubated with agitation for 1 hr at 37°C with goat-anti-rabbit IgG conjugated to alkaline phosphatase (Sigma-Aldrich) at 1:10 000 in TBS-T containing 1.5% milk. The membrane was washed 3-times for 10 min. each with TBS-T. The color was developed by incubation for 5-10 min. with liquid substrate contain nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Then membrane was washed with water and dried on air.

5. Gel electrophoresis of coat proteins

The purified virions of TMV-U_1, ToMV-2 and SHMV (2 mg of each virion in the sample) were dissociated by boiling for 3-5 min. in 60 mM Tris-HCl pH 6.8, 3% (w/v) sodium dodecyl sulfate (SDS), 6% mercaptoethanol, 10% glycerol and 0.005% bromophenol blue (McDaniel et al. 1995; Reddick 1989). Dissociated proteins was cooled in ice. Samples were separated on 12% polyacrylamide slab gel containing 0.1% SDS at 40-50V for 4 hrs. at room temperature. The protein bands were visualized by staining with 0.02% comassie brilant blue R-250 in 20% methanol and 10% acetic acid and destained with 20% methanol and 10% acetic acid or staining with silver. Size were estimated by comparision with size of b-lactoglobulin (18.4 kD), Trypsinogen (24 kD) and Albumin egg (45 kD).

6. Gel electrophoresis of whole virions

The whole virions were separated on 1% agarose gel in borate buffer pH 8 containing: 40 mM sodium borate, 0.25 mM EDTA, 0.25 M urea, at 25 V for 17 hrs. at 4°C (McDaniel et al. 1995; Reddick 1989). In each line were 50 mg of TMV-U_1, ToMV-2 and SHMV virions. After electrophoresis gel was stained with 0.02% coomassie brilant blue R-250 in 20% methanol and 10% acetic acid for 1 hr. and destained for 10hrs. with 20% methanol and 10% acetic acid.

III. RESULTS AND DISCUSSION

1. Host range and symptomatology

A summary of plant response to SHMV and TMV-U_1 is presented in Table. The majority of plant responses to inoculation by SHMV reported previously (Brunt et al. 1996) were confirmed by this study. Vein clearing, mosaic and malformation symptoms developed on noninoculated leaves of bean plants within 3 weeks after mechanical SHMV inoculation. SHMV, contrary to TMV did not cause small necrotic lesions on the inoculated leaves of bean plants 'Pinto'.

Following plant species allow to distinguish SHMV from TMV: *Phaseolus vulgaris* 'Fana' and 'Pinto', *Pisum sativum*, *Lupinus albus* and *Lycopersicon esculentum*. On these plant species both viruses produced different symptoms that clearly distinguish one from the other.

2. Electron microscopy and properties of purified virus

Rod-shaped virions, typical for tobamoviruses were detected in the crude sap of bean infected with SHMV and in purified preparation (Fig. 1). Average particles length for SHMV was about 300 nm but also shorter virions were found. In ultrathin section of bean leaves infected by SHMV bands of virions typical to tobamoviruses were observed (Fig. 2).

Table

Comparison of the host range and symptoms caused by SHMV and TMV-U_1

Plants	SHMV	TMV-U_1
Ch. album	LCh-NL; -	LNL; (Ss)
Ch. amaranticolor	LCh-NL; -	LChL; -
Ch. murale	LNL; -	LNL; (Ss)
Ch. quinoa	LChL; -	LChl; (Ss)
Lupinus albus cv. 'Wat'	-; SM	-; Ss
Lupinus luteus cv. 'Popiel'	-; SM, (s)	-; Ss
Lycopesicon esculentum cv. 'Betalux'	-; -	-, SM, SMa
N. affinis	LChL;Ss	LNL; STN
N. benthamiana	-; SM	Nt
N. glutinosa	LNL; -	LNL; -
N. tabacum cv. 'Samsun'	-; Ss	-; Sma; SChls
N. tabacum cv. 'Xanthi nc'	LNL, Ss	LNL; -
Phaseolus vulgaris cv. 'Fana'	LChL; SM; Sma	-; (Ss)
Pisum sativum cv. 'Meteor'	-; SM	-; (Ss)
Tetragonia expansa	LChL; -	-; -
Zinnia elegans	-; Ss	-; Ss

Symbols:
LNL = local necrotic lesions, **LChL** = local chlorotic lesions, **LCh-NL** = local chlorotic-necrotic lesions, **Ss** = systemic symptomless, **SM** = systemic mosaic, **SMa** = systemic malformations, **SChls** = systemic chlorotic spots, **TN** = top necrosis; – = no symptoms, ( ) = symptoms appered occasionally, **Nt** = not tested, **SHMV** = *Sunn hemp mosaic tobamovirus*, **TMV-U_1** = *Tobacco mosaic tobamovirus*, strain U_1

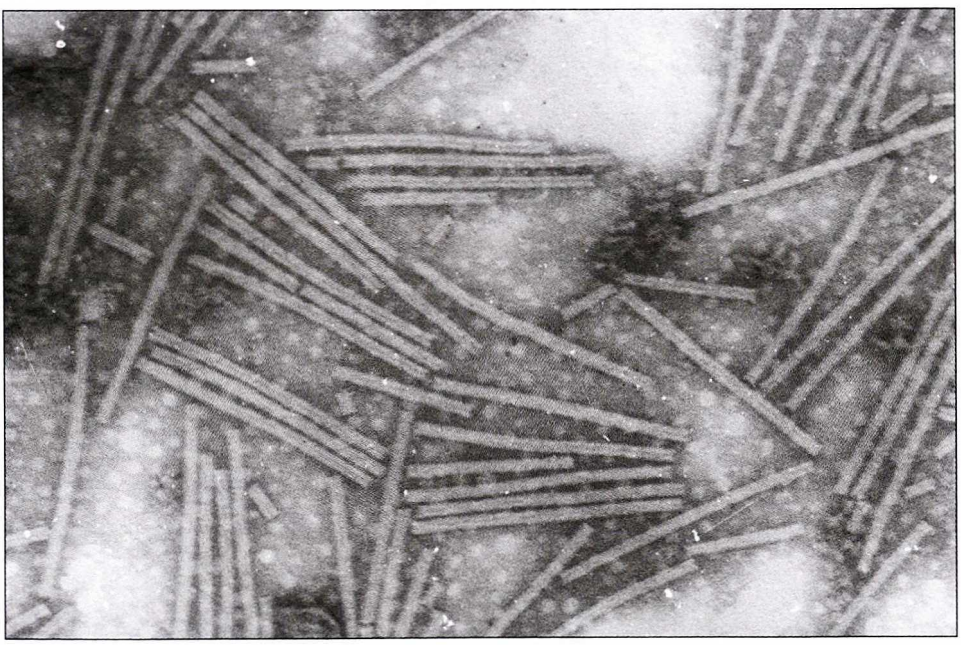

Fig. 1. Virions of SHMV in the been sap negativley stained with 2% PTA

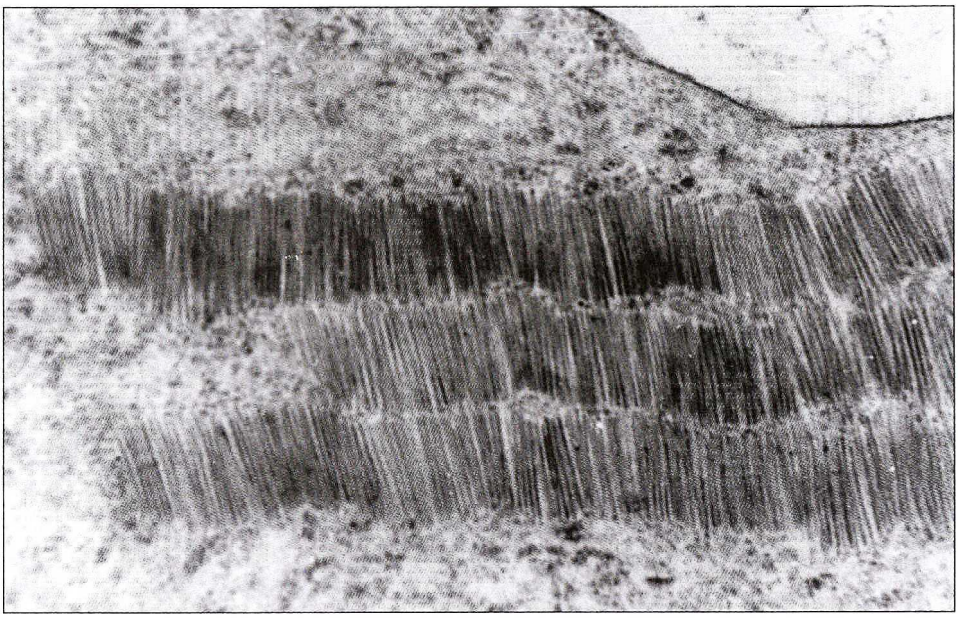

Fig. 2. Ultrathin section of *Phaseolus vulgaris* leaf infected by *Sunn-hemp mosaic tobamovirus* with characteristic bands of virions in the cytoplasm (x 41.000)

The yield of virus purified from bean plants was between 15-20 mg/100 leaves. Purified preparation showed UV absorption spectrum characteristics for nucleoproteins with an absorption maximum at 260 nm and the minimum at 247 nm. The ratio A_{260}/A_{280} was equalled 1.19.

3. Serology

The difference among examined strains of tobamoviruses was detected by immunoblotting. Antibodies against SHMV reacted strongly with two proteins of SHMV, but did not with proteins of ToMV-2 and TMV-U_1 (Fig. 3A). Antibodies to TMV-U_1 detected proteins of both ToMV and TMV-U_1 but did not react with SHMV (Fig. 3B). A distant serological relationship was reported between TMV and SHMV (Kassanis and Mc Carthy 1967), which capsid protein amino acid sequence differs by two-thirds from that of TMV (Rees and Short 1975).

4. Gel electrophoresis of virions and coat proteins

The whole virion electrophoresis showed individual profile of each virus. The biggest differences were observed between SHMV and other examined viruses. Virions of TMV-U_1 and ToMV-2 migrated as several bands and SHMV as two bands. The bands of ToMV-2 migrated always more rapidly then the bands of TMV-U_1 and the bands of virions of SHMV were the slowest (Fig. 4).

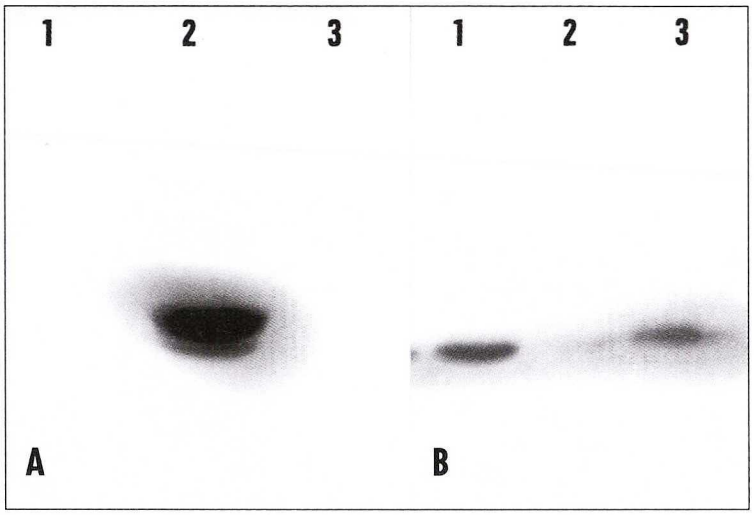

Fig. 3. The western-blot analysis of virion capsid proteins. Membranes were probed with polyconal antibodies to: (A) *Sunn hemp mosaic tobamovirusvirus* (SHMV), (B) *Tobacco mosaic tobamovirus* (TMV). Virion capsid proteins are from: TMV-U_1 (lane 1), SHMV (lane 2), and *Tomato mosaic tobamovirus* ToMV-2 (lane 3).

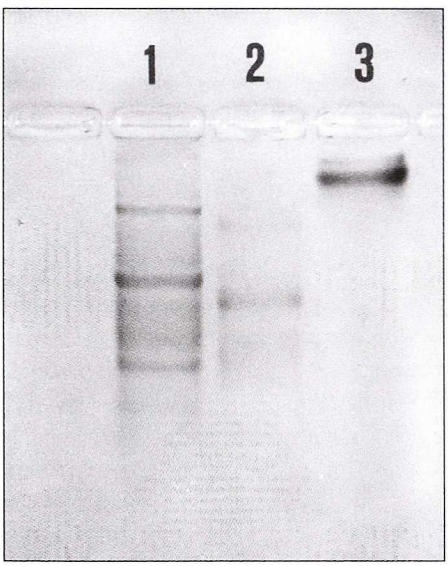

Fig. 4. Electrophoretic mobility of whole virions of TMV-U_1 (lane 1), ToMV-2 (lane 2) and SHMV (lane 3) in 1.5% agarose in sodium borate buffer at 4°C.

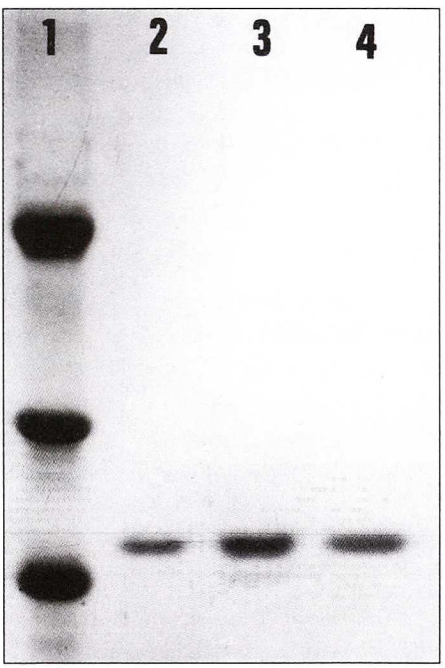

Fig. 5. Electrophoretic pattern of coat protein of purified virions in 12% polyacrylamide gel with 0.1% SDS. Lane 1, molecular weight standarts containing (from top to bottom) albumin egg (45 kD), trypsinogen (24 kD) and b-lactoglobulin (18.4 kD); Lane 2, TMV-U_1; Lane 3, SHMV and Lane 4, ToMV-2

The capsid proteins of TMV-U_1 and ToMV-2 migrated as a single bands having similar molecular weights (19.8 kD). SHMV migrated as two bands: 19.8 kD and 18.7 kD when resolved by SDS-PAGE (Fig. 5) as reported formerly by Goodwin et al. (1976).

IV. REFERENCES

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NIEKTÓRE WŁAŚCIWOŚCI WIRUSA MOZAIKI KROTALARII (*SUNN-HEMP MOSAIC VIRUS*) WYODRĘBNIONEGO Z ROŚLIN FASOLI (*PHASEOLUS VULGARIS* L.)

STRESZCZENIE

W pracy przedstawiono charakterystykę wirusa mozaiki krotalarii (SHMV) wyizolowanego z siewek fasoli wyrosłych z zainfekowanych nasion. Stwierdzono, że zakres roślin gospodarzy badanego izolatu SHMV oraz wywoływane przez niego objawy chorobowe były typowe dla tego wirusa. Gatunki roślin takie jak: *Phaseolus vulgaris*, *Pisum sativum*, *Lupinus albus* i *Lycopersicon esculentum* różnicują SHMV od TMV.

W teście immunoblotingu surowica przeciwko SHMV nie reagowała z TMV-U_1 i ToMV-2., z kolei surowica przeciwko TMV reagowała jedynie z TMV-U_1 i ToMV-2.

Obrazy elektroforetyczne zarówno całych virionów jak i białek otoczki wirusowej były charakterystyczne dla SHMV i różne od tych dla TMV-U_1 oraz ToMV-2.