

Acta Bot. Croat. 35 (1976) 33—39

SOME DATA ON SOWBANE MOSAIC VIRUS ISOLATED FROM *CHENOPODIUM MURALE* IN YUGOSLAVIA*

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Received December 24, 1975

Introduction

Sowbane mosaic virus (SMV) was first reported by Silva et al. (1958) who called it *Chenopodium* mosaic virus. Bennett and Costa (1961) discovered this virus originally in *Chenopodium murale* (sowbane) and gave it the name sowbane mosaic virus.

SMV represents a very well characterized virus (Kado 1967; Kado 1971; Kado and Blach 1968; Engelbrecht and van Regenmortel 1968; Paul and Huth 1970). This virus is interesting with regard to amino acid content because it differs in that respect from all known plant viruses (Kado 1966, 1967). SMV is a very stable virus with thermal inactivation point of about 90° C and dilution end point about 10⁻⁷. It is transmissible through seed in up to 83% of cases and it is very difficult to get virus free seedlings from seeds of infected plants (Diaz and Waterworth 1967; Kado 1971).

The virus occurs naturally in herbaceous plants, especially on species of *Chenopodiaceae* as well as in some woody plants. It is a wide spread virus and was reported from Australia, Japan, North and South America and S. Africa. In Yugoslavia SMV was first isolated from sour cherry (Šarić 1971). The same author found this virus in *Chenopodium quinoa* specimens which have been planted in a virus experimental greenhouse as test plants (Šarić 1969).

In this paper the identification of SMV originating from naturally infected *Chenopodium murale* in Yugoslavia will be presented.

* Dedicated to the memory of our late friend Dr. K. Schmelzer.

Material and Methods

The samples of naturally infected *Chenopodium murale* L. were collected in 1973 in field near Nova Gradiška (Yugoslavia). Among a great number of plants of this species only two specimens showed virus symptoms. The isolated virus was designated as SMV-J. For comparison an American isolate of SMV was also involved in our investigations. This virus was sent us by courtesy of Dr. H. E. Waterworth (Plant Introduction Station, Glenn Dale, USA) and it was designated in our experiments as SMV-A. Besides, the investigated SMV-J was serologically compared with a virus kindly supplied by Dr. A. Šarić (Faculty of Agriculture, Zagreb, Yugoslavia). This virus which was marked by us SMV-T belongs to SMV (Šarić, unpublished).

The SMV-J was partially purified after the chloroform-butanol method of Steere (1959) and the purified preparation was examined by means of electron microscope and spectrophotometrically.

The purified virus was used for immunization. Each rabbit received six intravenous injections, one injection per day. After the first three injections a three day pause followed. Every injection contained 1 ml of virus suspension of concentration at 1 mg/ml.

The serological experiments were carried out by means of the agar gel double-diffusion tests and the intragel absorption tests. The immunoelectrophoretic analyses were performed on an LKB electrophoresis apparatus (Type 6800A-1) with a procedure slightly modified (Juretić 1974) from that described by Hirschfeld (1960, 1962). The electrophoretic analyses took place at room temperature without cooling, under a potential of 8 V/cm for 4 hrs. Slides with antigens and immune sera were incubated for 24 hrs in a humid container at room temperature.

Fig. 1. Symptoms caused by the isolate SMV-J of sowbane mosaic virus. A, B — *Chenopodium murale* with mottling and mosaic. C, D *Chenopodium quinoa* with chlorotic zones, necrotic spots, curling and blistering of leaves.

Sl. 1. Simptomi zaraze koje uzrokuje izolat SMV-J virusa mozaika lobode. A, B — *Chenopodium murale* s pjegavošću i mozaikom. C, D — *Chenopodium quinoa* s klorotičnim područjima, nekrotičnim pjegama te kovrčanjem i mjehuravošću listova.

Fig. 2. Immunodiffusion tests (A, B, E, F), immunoelectrophoretic test (C) and virus particles (D). A, B — Gel diffusion precipitin patterns of SMV-J and SMV-A. C — immunoelectrophoretic migration of the mixture of SMV-J and SMV-A and separately SMV-A. D — Particles of SMV-J in a partially purified preparation. Magn. 100,000 x. E, F — Gel diffusion precipitin patterns of SMV-J and SMV-T. Abbreviations: A = SMV-A, J = SMV-J, T = SMV-T, sA = immune serum to SMV-A, sJ = immune serum to SMV-J.

Sl. 2. Imunodifuzijski pokusi (A, B, E, F), imunoelektroforetski pokus (C) i virusne čestice (D). A, B — precipitacijske linije u imunodifuzijskim pokusima u agarском gelu koje stvaraju SMV-J i SMV-A. C — imunoelektroforetska pokretljivost smjese SMV-J i SMV-A i odvojeno SMV-A. D — čestice SMV-J u djelomično purificiranom preparatu. Povećanje 100 000 x. E, F — precipitacijske linije u imunodifuzijskom pokusu u agarском gelu koje stvara SMV-J i SMV-T. Kratice: A = SMV-A, J = SMV-J, T = SMV-T, sA = imuni serum od SMV-A, sJ = imuni serum od SMV-J.

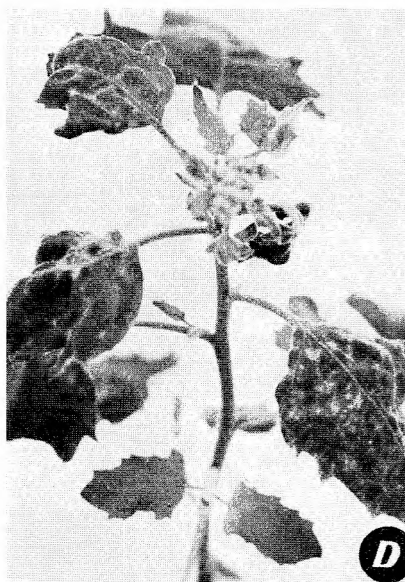
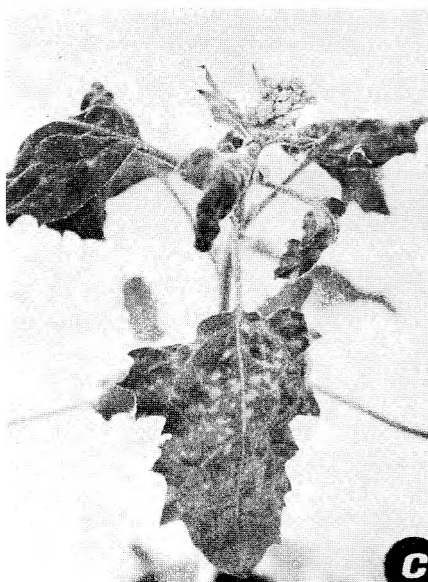
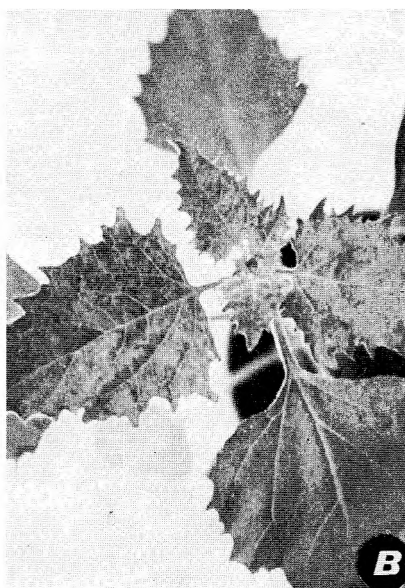
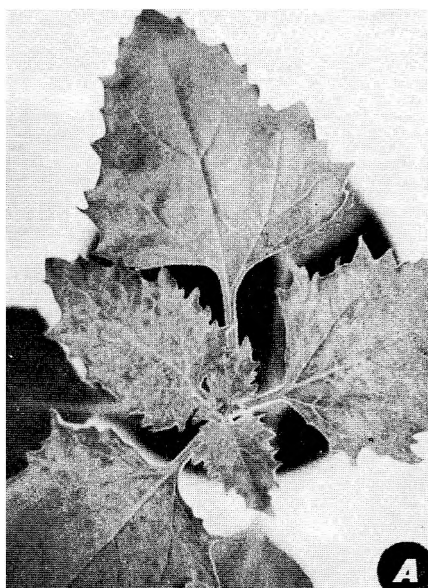


Fig. 1. — Sl. 1.

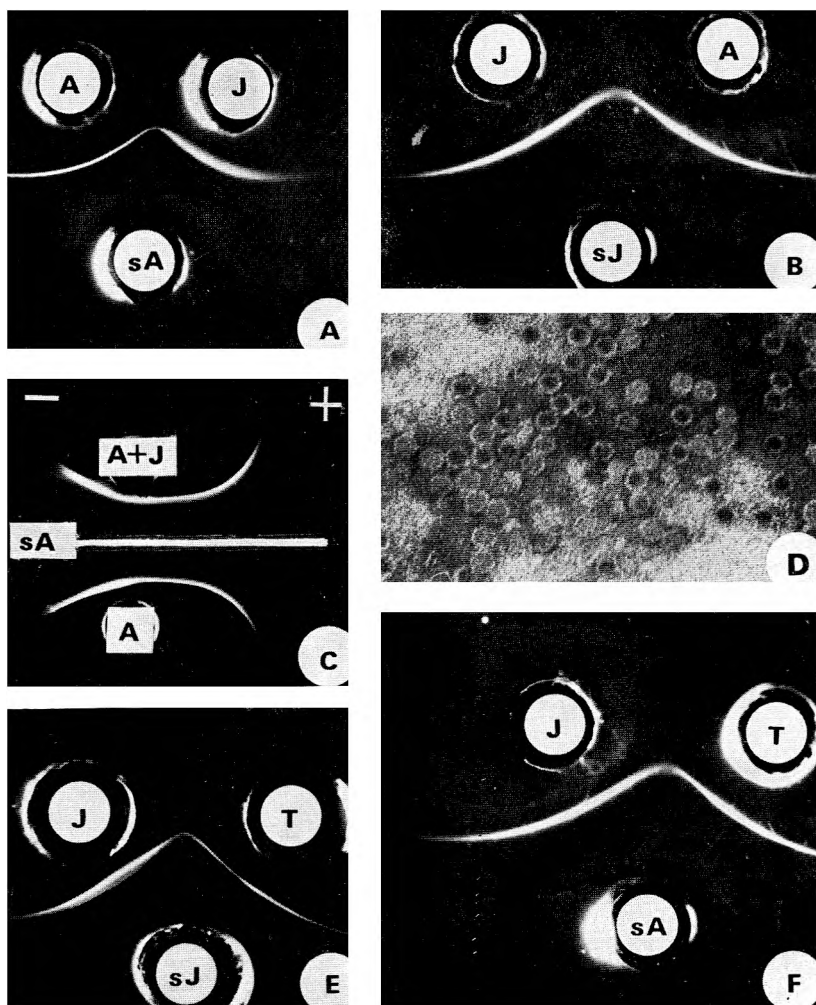


Fig. 2. — Sl. 2.

Results

Investigation on test plants

The isolate SMV-J was mechanically transmitted to 8 herbaceous test plants. Since the investigated isolate was found on *C. murale*, which represents a usual natural host of SMV, we presumed that our isolate belonged to SMV. Consequently test plants were mainly chosen from host range of SMV. Symptoms on these plants are shown in Table 1 and Fig. 1. In addition, the plants which did not react to SMV-J are also presented in Table 1. As it can be seen from the Table only plants from *Chenopodiaceae* are susceptible to the SMV-J. This is very well in concordance with the host range of SMV (cf. Bennett and Costa 1961). On the basis of reactions on test plants it was concluded that SMV-J could be an isolate of SMV.

Table 1. Reactions of test plants infected with isolate SMV-J of sowbane mosaic virus*

<i>Chenopodium album</i> L.	☐ I necrotic lesions; II milde mottling which disappear.
<i>C. amaranticolor</i> Coste et Reyn.	○ chlorotic or necrotic lesions.
<i>C. ambrosioides</i> L.	☆
<i>C. foetidum</i> Schrad.	☐ II mild mottling which disappears.
<i>C. foliosum</i> Aschers.	☐ II mild mottling which disappears.
<i>C. murale</i> L.	☐ II mottling, mosaic (Fig. 1a, b).
<i>C. quinoa</i> Willd.	☐ I necrotic lesions; II chlorotic zones, necrotic spots, curling, blistering (Fig. 1c, d).
<i>Spinacia oleracea</i> L.	☐ I chlorotic lesions; II mild variegation.
<i>Cucumis sativus</i> L.	△
<i>Datura stramonium</i> L.	△
<i>Nicotiana tabacum</i> L., cv. White Burley	△
<i>Nicotiana glutinosa</i> L.	△
<i>Ricinus communis</i> L.	△
<i>Rumex obtusifolius</i> L.	△
<i>Tetragonia expansa</i> (Pall.) O. Ktze.	△

* I = symptoms in inoculated leaves; II = symptoms in noninoculated top leaves; ○ = local infection; ☐ = systemic infection; ☆ = latent infection; △ = unsusceptible.

Investigations of physical properties

The following physical properties of SMV-J were established: thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV). The experiments showed that SMV-J had TIP about 93 °C, DEP about 10^{-7} and LIV more than 60 days (Fig. 3). From Fig. 3 it can be seen that during the first 30 days infectivity of the virus was unchanged. The above data are in concordance with the results reported for SMV by other authors (Benett and Costa 1961; Kado 1966; 1971). Consequently physical properties of SMV-J also indicated that it belonged to SMV.

Transmission through seed

32% of seed of the diseased sample of *Chenopodium murale* collected in field and then replanted in greenhouse produced infected seedlings. In the experiments the infectivity of the seedlings was controlled by mechanical inoculation of sap from each seedling in *Chenopodium quinoa* and serologically as well. The percentage of transmission of SMV-J through seed also indicated that SMV-J might belong to SMV.

Purification, ultraviolet absorption and electron microscopy

SMV-J was partially purified by the method of Steere (1959). Systematically infected leaves of *Spinacia oleracea* were used. The purified virus suspension had an absorption maximum in ultraviolet at 260 nm (Fig. 4). The ratio $A_{260/280}$ was 1.46. This number indicated that the SMV-J had approximately the ratio $A_{260/280}$ which was very close to that of the typical SMV (cf. Kado 1971).

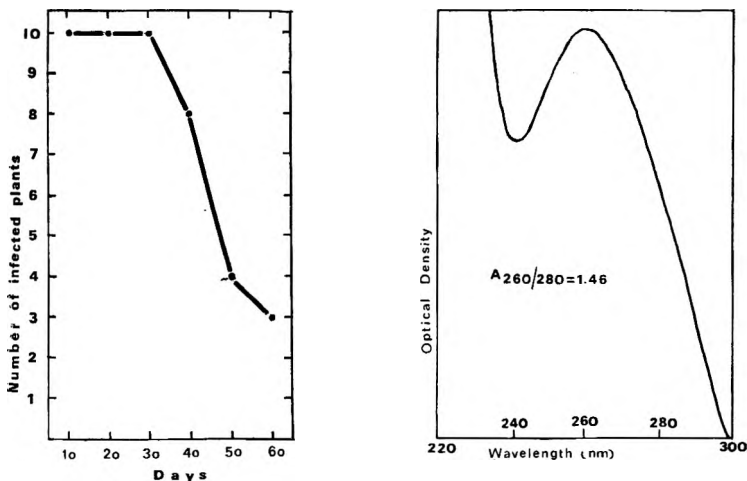


Fig. 3. Longevity in vitro of SMV-J of sowbane mosaic virus.

Sl. 3. Postojanost in vitro SMV-J virusa mozaika lobode.

Fig. 4. Ultraviolet absorption of purified isolate SMV-J of sowbane mosaic virus.

Sl. 4. Ultravioletna apsorpcija izolata SMV-J virusa mozaika lobode.

The analyses of partially purified SMV-J by electron microscope revealed isometric shaped virus particles about 30 nm in diameter (Fig. 2d), i. e. the virus particles of SMV-J could correspond to virus particles of SMV.

Serological investigations

No differences were detected between SMV-J and SMV-A in the agar gel diffusion tests although both the immune serum against SMV-A and the immune serum to SMV-J were used (Fig. 2a, b). It was also found that SMV-J did not differ from SMV-A in the experiments performed by the intragel cross absorption test. In addition, SMV-J was indistinguishable from SMV-A in the immuno electrophoretical mobility; it was found in experiments in which a mixture of SMV-J and SMV-A was analysed (Fig. 2c).

SMV-J was also compared serologically with SMV-T, which represents an isolate of SMV found in grapevine in Yugoslavia (see Material and Methods). In our serological experiments SMV-J and SMV-T were indistinguishable. These serological experiments were performed by means of immune sera against SMV-J and SMV-A (Fig. 2e, f).

Discussion

SMV-J infected 7 species of chenopods and also samples of *Spinacia oleracea* but failed to infect any of 7 other plant species from other families. *C. quinoa* and *C. murale* are better test plants for the investigated virus than other chenopods because they react regularly and with the most conspicuous symptoms. We have never observed these plants react in a latent way. These data agree with observations of Bennett and Costa (1961). The same authors found that *C. amaranticolor* reacted to SMV only with local symptoms. Also, in our experiments *C. amaranticolor* was not invaded systemically. During our investigations the majority of chenopods reacted systemically but often with symptoms which later disappeared (see Table 1).

SMV-J is in a relatively high percentage (32%) transmissible through seed. It must be pointed out that in our experiments only 50 seedlings grown from seed of infected plants were checked up in order to state the infectivity. However, 50 seedlings is too small a number to establish a real percentage of transmission through seeds.

Milne (1967) described dense, circular, granular regions in cells infected with SMV as observed in an electron microscope. We analysed the tissue of infected plants in a light microscope in order to find out whether SMV-J produces some light microscopically visible inclusion bodies. The analysis of *Spinacia oleracea* and *C. murale* infected tissue showed that SMV-J did not produce any inclusion bodies which could be visible by light microscope.

SMV was isolated so far from herbaceous as well as from woody plants (Kado 1971). Šarić (1971) considers that "it is probable that SMV is even more widespread on woody plants". This statement of the mentioned author seems to be possible because so far SMV was found on grapevine Bercks and Querfurth 1969; Šarić, personal communication), sour cherry (Šarić 1971) and on apple (Kirkpatrick et al. 1965; Bancroft and Tolin 1967).

Summary

A virus (SMV-J) in *Chenopodium murale* collected near Nova Gradiška (Yugoslavia) was found to be an isolate of sowbane mosaic virus (SMV). The virus was transmitted to 8 species of *Chenopodiaceae* but it failed to infect 7 species of plants from other families. The virus was isolated and purified. Electron microscope analyses revealed isometric virus particles about 30 nm in size. SMV-J was transmitted readily through seed; 32% of the seeds of diseased plants produced infected seedlings.

The virus has a thermal inactivation point at about 93 °C. It remained active in vitro more than 2 months. An immune serum against SMV-J was prepared. The serological tests performed by the agar gel double diffusion test and the intragel absorption test showed that SMV-J is indistinguishable from the American strain of SMV. In addition, SMV-J did not differ serologically from SMV isolate found in grapevine in Yugoslavia.

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My thanks are due to Dr. A. Šarić (Faculty of Agriculture, Zagreb) who kindly supplied some isolates of sowbane mosaic virus. The author thanks Dr. N. Ljubešić for electron microscopy, and Miss T. Drajer for technical help.

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S A D R Ź A J

NEKI PODACI O VIRUSU MOZAIKA LOBODE IZOLIRANOM IZ VRSTE *CHENOPODIUM MURALE* U JUGOSLAVIJI

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Na primjercima vrste *Chenopodium murale* koji su sabrani u okolici N. Gradiške nađen je virus (SMV-J), koji predstavlja izolat virusa mozaika lobode (sowbane mosaic virus, SMV). Virus je prenijet na osam vrsta iz porodice *Chenopodiaceae*, ali ga nismo uspjeli prenijeti na sedam vrsta koje pripadaju drugim biljnim porodicama. Virus je izoliran i purificiran prema metodi Steere (1959). Elektronskomikroskopskom analizom purificirane virusne suspenzije nađene su izometrične virusne čestice promjera oko 30 nm. SMV-J lako se prenosi sjemenom vrste *C. murale*. Iz 32% sjemenaka, sabranih u polju na inficiranoj biljci, nikle su zaražene biljke. Termalna točka inaktivacije istraživanog virusa je oko 93°C, a može postojati in vitro više od 2 mjeseca. Krajnja točka razrjeđenja mu je oko 10⁻⁷.

Protiv SMV-J priređen je imuni serum. Serološki pokusi izvedeni metodom dvostruke difuzije u agarском gelu te metodom unakrsnog zasićavanja u agaru pokazali su da se SMV-J ne razlikuje od američkog soja virusa mozaika lobode (SMV-A). On se od SMV-A ne razlikuje niti po elektroforetskoj pokretljivosti. Osim toga SMV-J se serološki ne razlikuje niti od izolata SMV koji je nađen u vinovoj lozi u Jugoslaviji.

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