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BELLADONNA MOTTLE VIRUS IN YUGOSLAVIA

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Introduction

Belladonna mottle virus (BMV) was described for the first time in Germany by Bode and Marcus (1959) and was determined as a distinct virus by Paul, Bode, Jankulowa and Brandes (1968). The virus is a member of tymoviruses and belongs to the Andean potato latent subgroup (Paul et al. 1968; Jankulowa, Huth, Wittmann and Paul 1968). Its cryptogram is R/1: 2.0/37 : S/S : S/Cl. Until recently the virus has been known only from Germany and Bulgaria (Paul 1971). During last year we isolated and identified BMV in Yugoslavia. The identification was based on test plants reactions and serology.

Material and Methods

The stock culture of BMV (isolate BMV-M) was obtained from belladonna plant (*Atropa belladonna* L.) collected in the Medvednica Mountain near Zagreb and kindly supplied by Professor D. Miličić. The leaves of the infected plant exhibited vein-banding, i. e. a symptom typical of those from which BMV was isolated earlier (Paul et al. 1971; Abb. 1). The virus was transmitted by manual inoculation of crude sap to test plants and was maintained in *Nicotiana glutinosa*.

Serological tests were made by double diffusion tests in 0.9% agar-gel containing 0.9% NaCl and 0.02% NaN_3. For this reactions crude infective sap was clarified by low-speed centrifugation (3000—4000 rpm).

A culture of original BMV isolate (BMV 2) (Paul et al. 1968) and serum to BMV were kindly supplied by Dr H. L. Paul, Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig.

Results

Occurrence and separation of the virus

During 1973 belladonna plants with virus-like symptoms were noticed at different sites in the Medvednica Mountain near Zagreb. The leaves of the plants showed either vein-banding or mosaic. Sometimes about 50% of the specimens exhibited the symptoms of infection.

The specimen from which BMV-M originated was infected in addition with another virus. Light microscopy of the test plants which had been infected with the sap from the original plant revealed numerous hexagonal prisms in the cytoplasm. Inclusion bodies in the form of hexagonal prisms are characteristic of tobacco mosaic virus (TMV) and allied tomato mosaic virus (ToMV) (Miličić 1969); however, the inoculum caused only local lesions in inoculated leaves of *Chenopodium amaranticolor* and this suggested the presence of TMV because ToMV infects this plant systemically (Juretić 1971).

BMV-M was obtained free from the mixture with TMV by use of *Nicotiana glutinosa* in which, if grown at temperature below 32° C, TMV remains localized in inoculated leaves and BMV passes to tip leaves. The tip leaves of *N. glutinosa* contained neither the hexagonal prisms nor did their sap cause local lesions in *Ch. amaranticolor*.

Symptoms in test plants

By means of the light microscope it was established that plastids of infected belladonna were strongly vesiculated. Owing to these changes it was supposed that the virus belonged to BMV. Namely, similar vesiculation of the plastids under the influence of BMV was described by Harrison and Roberts (1970). Therefore BMV-M was inoculated to a range of solanaceous species which are hosts of BMV. It was transmitted to 7 species of the *Solanaceae*.

Fig. 1. (A), (B) and (C). *Nicotiana tabacum White Burley*. Different types of systemic symptoms caused by BMV-M isolate of belladonna mottle virus (BMV). (A) Fine vein-clearing, (B) veinal necrosis, (C) leaf deformations.
(D). Mottling and mosaic in *N. glutinosa* infected with BMV (isolate BMV-M).
(E) and (F). Immunodiffusion tests of leaf extracts containing BMV 2 isolate (2) and BMV-M isolate (M) with serum against BMV (s BMV) or serum which has been absorbed with BMV-M isolate (M abs s BMV). Serum dilution 1/16.

Sl. 1. (A), (B) i (C). *Nicotiana tabacum White Burley*. Razni oblici sistemičnih simptoma izazvanih BMV-M izolatom virusa šarenila velebilja (VŠV). (A) Fino prosvjetljivanje nerava, (B) nekroze nerava, (C) deformacije lista.
(D). Mozaično šarenilo na vrsti *N. glutinosa* zaraženoj s VŠV (BMV-M izolat).
(E) i (F). Imunodifuzijski pokusi s ekstraktima listova koji sadrže BMV 2 izolat (2) i BMV-M izolat (M) i serumom protiv VŠV (s BMV) ili serumom koji je bio absorbiran pomoću BMV-M izolata (M abs s BMV). Razrjeđenje seruma 1/16.

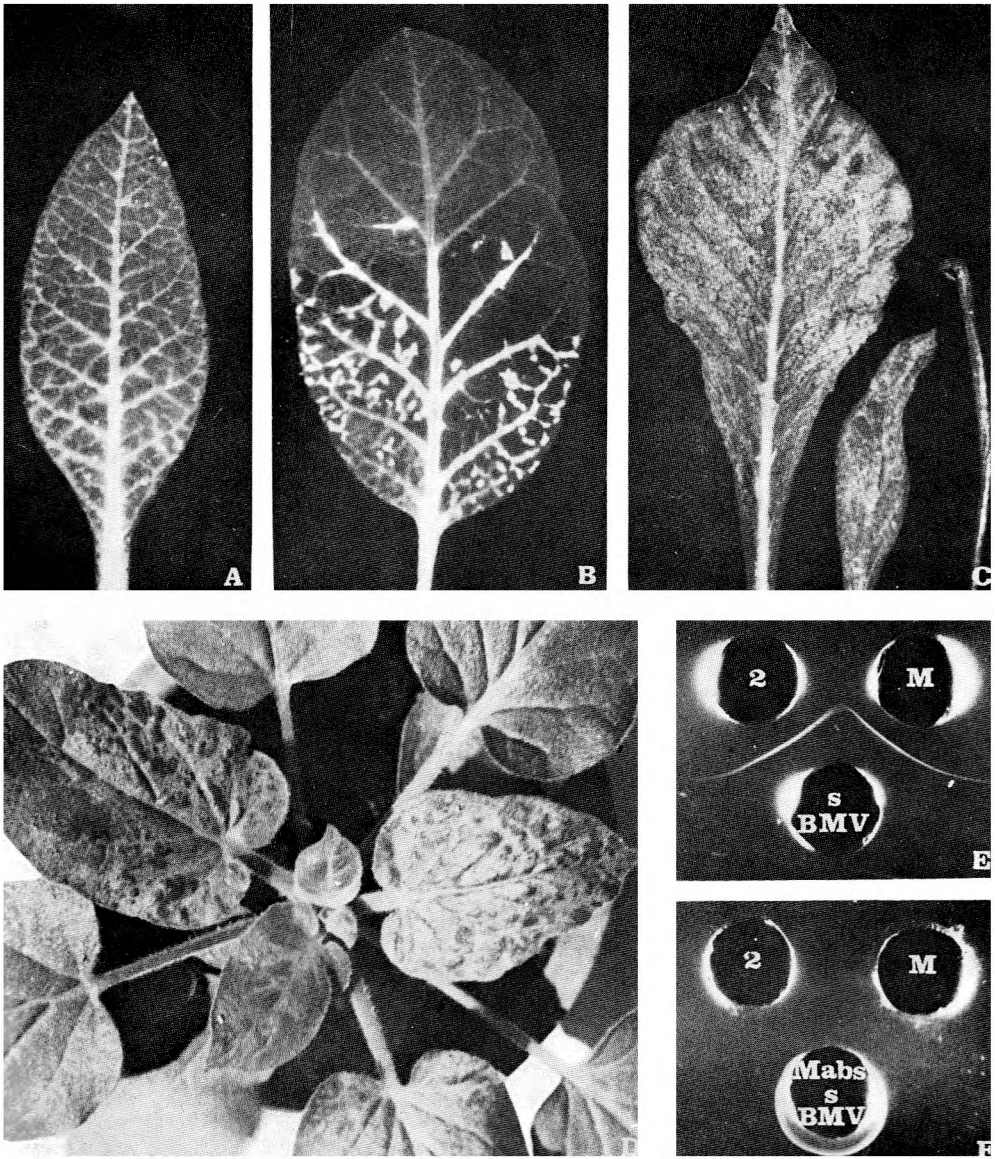

Fig. 1. — Sl. 1.

The symptoms produced in tobacco (*Nicotiana tabacum* L. "Samsun" and "White Burley") were distinctive and, as shown earlier (Paul et al. 1968), varied considerably depending on weather conditions. Inoculated leaves developed numerous chlorotic or necrotic local lesions often joined already at the beginning. Systemically infected leaves developed vein-clearing (Fig. 1 A) followed by veinal necrosis (Fig. 1 B). Later the tip leaves showed mosaic, mottling and general necrosis. A group of the plants which were infected during the winter exhibited conspicuous leaf deformation (Fig. 1 C) not earlier described for BMV, the symptom being well-known for cucumber mosaic virus. The sap from these plants was inoculated twice in *Chenopodium quinoa* and some other *Chenopodium* species but neither local lesions, which indicate infection with cucumber mosaic virus, nor any other symptoms of virus infection appeared.

The rest of the test plants developed fairly similar symptoms as tobacco did. The reactions in these species are given in Table 1.

Table 1. Test plants and symptoms of BMV-M

Atropa belladonna L.	After 12—14 days in tip leaves vein-clearing followed by mosaic, mottling and vein-banding.
Datura stramonium L.	Numerous necrotic or chlorotic local lesions after 5—7 days. Systemic infection follows soon with mosaic, chlorotic spots and necrosis.
Lycopersicon esculentum Mill.	Numerous necrotic local lesions after 5—7 days, followed by systemic mosaic and/or necrotic line pattern.
Nicotiana clevelandii Gray.	Many necrotic local lesions in the inoculated leaves; vein-clearing followed by veinlet necrosis or chlorotic spots in the systemically infected leaves.
N. glutinosa L.	Symptoms similar to those in tobacco but with less leaf deformation. Frequent mottling (Fig. 1 D).
N. megalosiphon Heurck et Muell.	Chlorotic or necrotic local lesions; mottling and necrosis in tip leaves.
N. tabacum (Samsun and White Burley)	(symptoms described in text).

Changes in plastids

Plastids in leaf epidermal cells of belladonna and other experimental plants infected with BMV-M developed distinct vesicles. Vesiculated plastids of a cell fused sometimes together building a large body similar in its appearance under the light microscope to the amorphous inclusions of some other viruses. The changes described in the plastids resembled those caused by turnip yellow mosaic (Miličić and Štefanac 1967), belladonna mottle or eggplant mosaic (Harrison and Roberts 1970) viruses. This effect on plastids seems to be characteristic of the group of tymoviruses.

Serological tests

The serum against BMV provided by Dr H. L. Paul had a titre of 1/4096 in gel-diffusion tests with clarified preparations of BMV 2 or BMV-M. When the viruses were placed in adjacent wells and tested

with BMV serum, the coalescence of precipitin lines appeared without forming a spur (Fig. 1 E). In intra-gel absorption tests, the BMV serum after absorption with BMV-M did not react either with BMV-M or with BMV 2 (Fig. 1 F). Consequently, gel-diffusion tests showed that the two viruses are serologically identical.

Discussion

A virus disease of belladonna (*Atropa belladonna* L.) was described in Yugoslavia by Perišić (1953). According to the symptoms on belladonna and test plants that virus was found to be similar to belladonna mosaic virus which was subsequently identified as tobacco rattle virus (Paul 1971).

In the present work it has been established that another virus, i. e. BMV, exists in belladonna plants in Yugoslavia. The investigations of our isolate have shown that it is serologically identical with BMV 2 isolate described by Paul et al. (1968). This result is in agreement with previous findings of Jankulowa et al. (1968) who found that different isolates of BMV were almost identical in their properties, despite of their different geographical origin. However, it is to be said that all these isolates originated from belladonna. Recently Moline (1973) described BMV from Physalis heterophylla in Iowa (USA) which he found to be distinct serologically from the type strain from belladonna.

It seems that belladonna plants in the surroundings of Zagreb are frequently infected with BMV because specimens with characteristic symptoms of BMV have been noticed repeatedly and in a high percentage on many localities in the Medvednica Mountain near Zagreb.

Summary

Belladonna mottle virus, isolated from belladonna (*Atropa belladonna* L.), was found in Yugoslavia for the first time. The BMV-M isolate from belladonna produced symptoms typical of belladonna mottle virus in test plants, and also conspicuous deformations of leaves in tobacco. In double diffusion tests the isolate was found to be serologically identical with BMV 2 isolate described from belladonna in Germany (Paul et al. 1968).

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SADRŽAJ

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Virus šarenila velebilja pronašli smo prvi put u Jugoslaviji, i to na velebilju (*Atropa belladonna* L.). Simptomi koje je na pokusnim biljkama stvarao izolat BMV-M bili su karakteristični za virus šarenila velebilja. Međutim, u duhanskih biljaka sorte White Burley pored tih karakterističnih simptoma zapažale su se i razne deformacije lista. U pokusima dvostruke difuzije u agaru izolat BMV-M pokazao je serološku identičnost s izolatom BMV 2, koji je prvotno bio izoliran iz velebilja u Njemačkoj (P a u l i sur. 1968).

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