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Natural Occurrence of Belladonna Mottle Virus in Hungary¹⁾

By

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With 7 figures

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Introduction

Belladonna mottle virus (BMV, cryptogram: R/1 : 2.0/37 : S/S : S/Cl) was found in spontaneously infected *Atropa bella-donna* L. plants in Hungary. Identification, together with some properties of the virus, are reported in the present paper. This virus with isometric particles of c. 27 nm in diameter, a member of the turnip yellow mosaic virus (TYMV) group, (tymovirus group) was first reported by BODE and MARCUS (1959) in Germany and determined as a distinct virus by PAUL et al. (1968), JANKULOWA et al. (1968), PAUL (1969, 1971). By now the virus has also been reported from Bulgaria (PAUL et al. 1968), United States of America (MOLINE and FRIES 1972, 1974, PETERS and DERKS 1974) and Yugoslavia (ŠTEFANAC 1974). According to the recent comprehensive study of serological relationships among tymoviruses (cf. JANKULOWA et al. 1968, BERCKS and QUERFURTH 1971, KOENIG and GIVORD 1974) BMV is more closely related serologically to dulcamara mottle virus (DMV), Andean potato latent virus (APLV) and eggplant mosaic virus (EMV) than to other tymoviruses. On the basis of previous serological subdivision of tymoviruses into two subgroups (cf. GIBBS et al. 1966) BMV has been allied to APLV subgroup (cf. HARRISON et al. 1971).

Spontaneous infections with BMV have been recorded in solanaceous plants only. Besides BMV, described in this paper, another member of the tymovirus group, TYMV, recently has been found in Hungary (JURETIĆ et al. 1973, HORVÁTH et al. 1973).

¹⁾ In memoriam Dr. sc. K. SCHMELZER († 28. May 1976).

Table 2

New resistant plants of H isolate of belladonna mottle virus isolated from *Atropa bella-donna* in Hungary

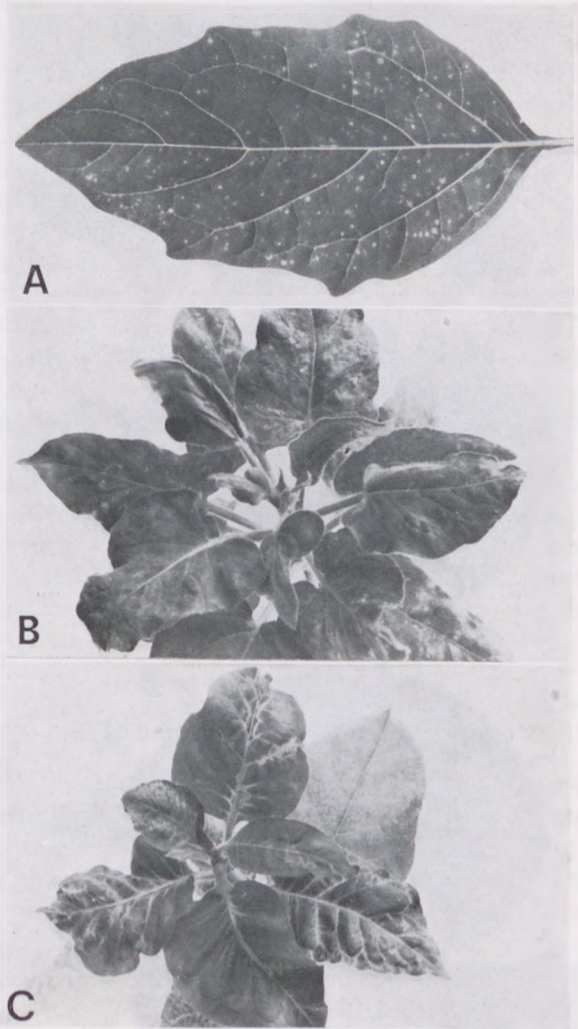
<i>Ammi majus</i> L.	<i>Helianthus salicifolius</i> A. Dietr. ^o
<i>A. visnaga</i> (L.) Lam. ^o	<i>H. tomentosus</i> Mchx. ^o
<i>Beta lomatogona</i> Fisch. et Mey.	<i>H. trachelifolius</i> Mill. ^o
<i>Chenopodium amaranticolor</i> Coste et Reyn.	<i>Phaseolus lunatus</i> L.
<i>Cucurbita pepo</i> L. convar. <i>patissonia</i> Greb.	<i>P. vulgaris</i> L. cv. Pinto
<i>f. radiata</i> Nois. ^o	<i>P. vulgaris</i> L. cv. Red Kidney
<i>Erodium ciconium</i> (L.) L'Hérit. ex Ait. ^o	<i>Rhoeo discolor</i> Hance
<i>E. gruinum</i> (L.) L'Hérit. ex Ait.	<i>Rorippa islandica</i> (Oeder) Borb.
<i>E. malacoides</i> Willd. ^o	<i>Silene armeria</i> L.
<i>E. manescavi</i> Coss. ^o	<i>S. conica</i> L. ^o
<i>E. moschatum</i> (L.) L'Hérit. ex Ait.	<i>S. dichotoma</i> Ehrh.
<i>Geranium pyrenaicum</i> Burm. ^o	<i>S. gallica</i> L. ^o
<i>G. robertianum</i> L.	<i>S. pendula</i> L.
<i>G. rotundifolium</i> L.	<i>S. tatarica</i> (L.) Pers. ^o
<i>G. sibiricum</i> L. ^o	<i>Solanum demissum</i> Lindl. A6-hybrid
<i>Gomphrena decumbens</i> Jacq. ^o	<i>S. demissum</i> Lindl. Redd. 530-hybrid
<i>Helianthemum nummularium</i> (L.) Dun.	<i>S. demissum</i> Lindl. Stamm S-hybrid
<i>Helianthus atrorubens</i> L. ^o	<i>Tetragonia crystallina</i> L'Hérit. ^o
<i>H. californicus</i> DC. ^o	<i>T. echinata</i> Ait.
<i>H. cernuus</i> Benth. et Hook. ^o	<i>Tropaeolum minus</i> L.
<i>H. decapetalus</i> L. ^o	<i>T. peltophorum</i> Benth. ^o
<i>H. doronicoides</i> Lam. ^o	<i>T. peregrinum</i> L.
<i>H. giganteus</i> L. ^o	<i>Vaccaria segetalis</i> (L.) Scop. ^o
<i>H. grosse-serratus</i> Mart. ^o	<i>Vigna sinensis</i> L. var. <i>sesquipedalis</i> (L.)
<i>H. maximiliani</i> Schrad. ^o	Van Eselt. ^o
<i>H. mollis</i> Lam. ^o	<i>Viscaria vulgaris</i> Bernh.
<i>H. organophyllus</i> Torr. et Gray. ^o	

^o Plant species are new experimental plants in plant virology.

When studying in detail the host range of isolate H, we found that the German isolate and PMV hosts pointed out by PAUL et al. (ibid.) and PETERS and DERKS (ibid.), responded to inoculation with isolate H with the same symptoms as those described by PAUL et al. (ibid.) and PETERS and DERKS (ibid.). Exceptions to this were *Cyphomandra betacea* Standt., *Datura stramonium* var. *gordonii*, *Hyoscyamus niger* L., *Nicotiana tabacum* cv. F80, *Solanum nigrum* L. var. *nodiflorum*, *S. nigrum* var. *villosum*, which had not been included in our own experiments. Plants resistant to the H isolate (*Beta vulgaris* L., *Brassica rapa* L., *Chenopodium album* L., *C. quinoa* Willd., *Cucumis sativus*, *Gomphrena globosa* L., *Melandrium album* [Mill.] Garcke, *Phaseolus vulgaris* L. cv. Red Kidney, *Solanum demissum* Lindl., *S. tuberosum* L., *Spinacia oleracea* L., *Tetragonia expansa* Murr. [syn.: *T. tetragonoides* (Pall.) O. Ktze], *Vicia faba* L., *Vigna sinensis* [L.] Endl., *Zinnia elegans* Jacq.) were found to be resistant in the experiments of PAUL et al. (ibid.) and by PETERS and DERKS (ibid.).

We have to note here that we could not point out a latent local virus susceptibility to isolate H for *Chenopodium quinoa* found to be a latent local

Fig. 2. Reactions of various test plants to the infection with H isolate of belladonna mottle virus. A: *Datura stramonium* L. (local lesions), B: *Nicotiana glutinosa* L. (local and systemic symptoms), C: *Nicotiana tabacum* L. cv. Xanthi-nc (systemic symptoms)



host in the experiments of PAUL et al. (ibid.) and local host to *Physalis mottle virus* (PMtV) isolated from *Physalis heterophylla* L. in Central Iowa (MOLINE and FRIES 1972, 1974); this is supposed to be due to inhibitors in *C. quinoa*. There is, however, an interesting difference between the H isolate and the German isolate as well as the PMV; namely *Atropa bella-donna*, a plant resistant to PMV, was found to be not only a natural but also an artificial host to H isolate.

The large number of corresponding results obtained in our experiments and in those carried out by PAUL et al. (1968) also suggested that the H isolate was very similar to or identical with the German isolate. While studying the host range we pointed out 48 new hosts (see table 1, fig. 3) and 51 resistant plants (table 2). Of the 99 plants tested 50 are new ones in the literature on plant virology.

As mentioned in "Introduction" the only spontaneous hosts of BMV are found among solanaceous plants. Just three species outside the *Solanaceae*, i. e. *Chenopodium quinoa* (PAUL et al. 1968, MOLINE and FRIES 1974), *C. foetidum* Schrad. and *Sonchus oleraceus* L. (PETERS and DERKS 1974) have been found as being susceptible to the isolates of that virus. This paper brings out evidence on a new experimental host of BMV outside the *Solanaceae*, notably *Hibiscus manihot* L. (*Malvaceae*). The finding is of some interest as recently an ad-

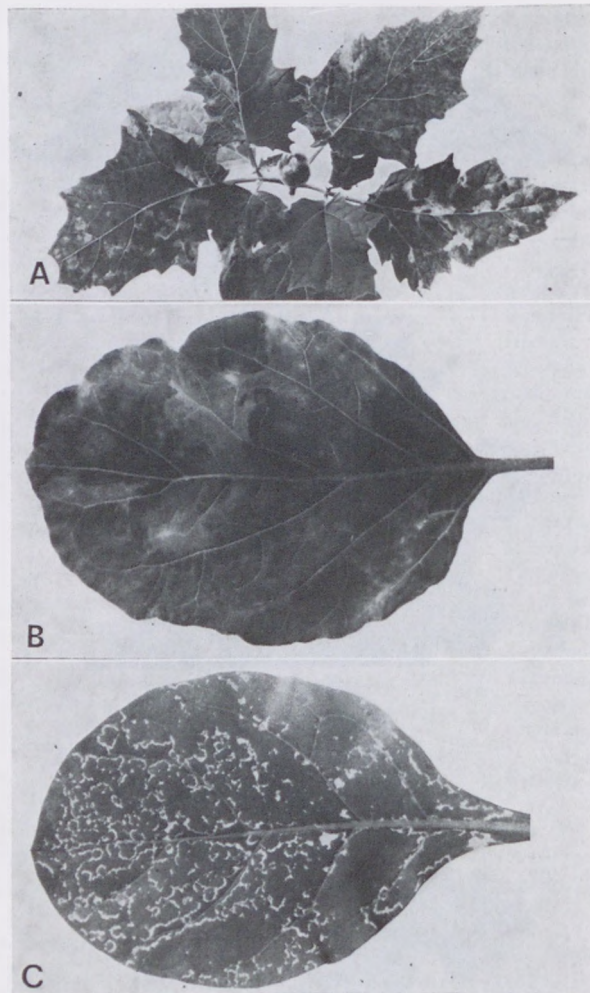


Fig. 3. Reactions of various new host plants to the infection with H isolate of belladonna mottle virus. A: *Datura godronii* Danert cv. Minka (systemic symptoms); B: *Nicotiana paniculata* L. (systemic symptoms) and C: *Nicotiana auriculata* Bert. (local symptoms)

ditional member of tymovirus group, i. e. okra mosaic virus (OMV) was recorded in *Hibiscus* sp. (cf. GIVORD and KOENIG 1974). There is also some evidence that BMV can cause infection in certain *Digitalis* spp. in the family *Scrophulariaceae* (cf. MAMULA 1976).

2. Transmission

Myzus persicae failed to transmit the H isolate (similar to the German isolate, cf. PAUL et al. 1968, WEIDEMANN and BODE 1973) either in styletborne or in circulative manner. The afore-

mentioned authors found, in addition, that BMV-"type" could be transmitted through seed. It has been shown that BMV-"type" could be transmitted through flea-beetles, *Epithrix atropae* Foundr. (JANKULOWA et al. 1968, WEIDEMANN and BODE 1973).

3. Serology

The H isolate gave a positive serological reaction with an antiserum (titre c. 1 : 256) against a Yugoslav isolate of BMV (ŠTEFANAC 1974, MAMULA 1976). It gave precipitation to the same antiserum dilution as the homologous virus did. Moreover, in straight gel diffusion (spur test) and intragel absorption experiments the H isolate could not be distinguished serologically from the Yugoslav isolate of BMV, using antiserum to the latter (fig. 4). These

examinations proved beyond doubt a close serological similarity of the H and Yugoslav isolates.

Serological investigations carried out before now with isolates of BMV indicated that the European isolates are closely related to each other, but those from the USA differ bad phrase from them. The German and the Bulgarian isolates were very similar serologically (cf. JANKULOWA et al. 1968), and it appeared that Yugoslav isolate was indistinguishable serologically from the German isolate (ŠTEFANAC 1974). As it is shown in the present paper the H isolate was indistinguishable from the Yugoslav isolate.

It is to be noticed that the serological relationship between the Yugoslav and German isolates was examined unilaterally, that is, by means of antiserum to the German isolate only (ŠTEFANAC 1974). Similarly in the present paper with isolates H and Yugoslav, only antiserum to the Yugoslav isolate was applied. It is worthwhile to mention that these four European isolates were recovered from the natural host, *Atropa bella-donna*. On the other hand, the two American isolates of PMV and PMtV, which originated from *Physalis* spp., differed distinctly from those of Europe, notably from the German isolate. As regards this, PMtV was somewhat more distant serologically than BMV from the German isolate of BMV (cf. MOLINE and FRIES 1974, PETERS and DERKS 1974). It is of some interest to mention that the H isolate used as the antigen in experiments was taken from naturally infected *Atropa bella-donna* plants.

4. Cell alterations

Leaf cells of *Nicotiana glutinosa* and *N. megalosiphon* displayed chloroplast abnormalities due to infection by the H isolate of BMV. First, one to several vacuoles appeared in a single chloroplast leading to the enlargement of the chloroplast. Chloroplasts then simultaneously formed groups, with the apparent disintegration of the individual chloroplasts. The altered chloroplasts

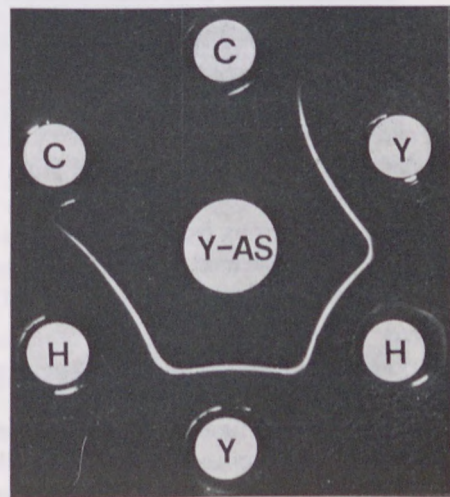


Fig. 4. Agar-gel double diffusion test with belladonna mottle virus isolates. The central well (Y-AS) was filled with the antiserum of Yugoslavian belladonna mottle virus isolate, the H wells contained the Hungarian belladonna mottle virus isolate from *Atropa bella-donna* L., the Y wells contained the Yugoslavian belladonna mottle virus isolate from *Atropa bella-donna* L., and the C wells contained healthy plant sap, respectively

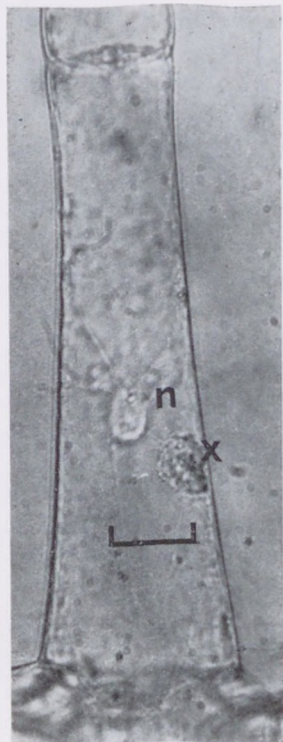


Fig. 5. Leaf marginal hair cells of *Nicotiana glutinosa* L. containing cytoplasmic inclusion body of H isolate of belladonna mottle virus. n, nucleus; x, inclusion body. Bar represents 20 μ m

lost their characteristic structure and became highly vesiculated (fig. 5). The process of chloroplast discoloration was also obvious. Vacuole formation in the chloroplasts was particularly conspicuous in the subepidermal cells of leaves. This type of cytological change, i. e. chloroplast alteration and presence of specific cytoplasmic inclusions in plants infected with the H isolate, seems thus to be characteristic of BMV (cf. MOLINE 1973, ŠTEFANAC 1974). Such alterations have also been found in some other tymoviruses, notably in TYMV (cf. RUBIO 1956, GEROLA et al. 1966, CHALCROFT and MATTHEWS 1967, MILIĆIĆ and ŠTEFANAC 1967), EMV (HARRISON and ROBERTS 1970) and wild cucumber mosaic virus (WCMV, ALLEN 1972). Recently it was reported (MOLINE 1973) that cells of *Datura stramonium* infected with PMtV, a strain of BMV, contained crystalline aggregates of the virus in the cytoplasm

and in the nucleus. The presence of viral crystals in the nucleus is interesting as it is uncommon with tymoviruses. This phenomenon is, however, characteristic of some viruses such as tobacco etch virus (TEV, potyvirus group; cf. SHEPHARD and PURCIFULL 1971).

5. Purification, ultraviolet absorption and electron microscope analysis

Partially purified preparations obtained after two cycles of differential centrifugation contained a small quantity of impurities such as phenols, and revealed a strong opalescence. They showed ultraviolet absorption characteristic of nucleoprotein (fig. 6). Virus concentration in the crude sap was estimated as 0.4 g/l as calculated from spectrophotometric data.

Electron micrographs of partially purified preparations of the H isolate displayed numerous isometric virus particles averaging c. 28 nm in diameter. Many particles seemed to be empty protein shells. Particles showed a tendency of aggregation in a crystalline array (fig. 7).

6. Physical properties

The thermal inactivation point of the H isolate ranged from 76 °C to 78 °C. The dilution end-point was between 2×10^{-6} and 10^{-6} and the longevity *in vitro* (at c. 20 °C) was 15 days.

Fig. 6. Ultraviolet absorption spectrum of partially purified H isolate of belladonna mottle virus. Note maximum absorption at c. 260 nm. $A_{260/280} = 1.63$. Spectrophotometer Model: Varian Teshtron 635 D

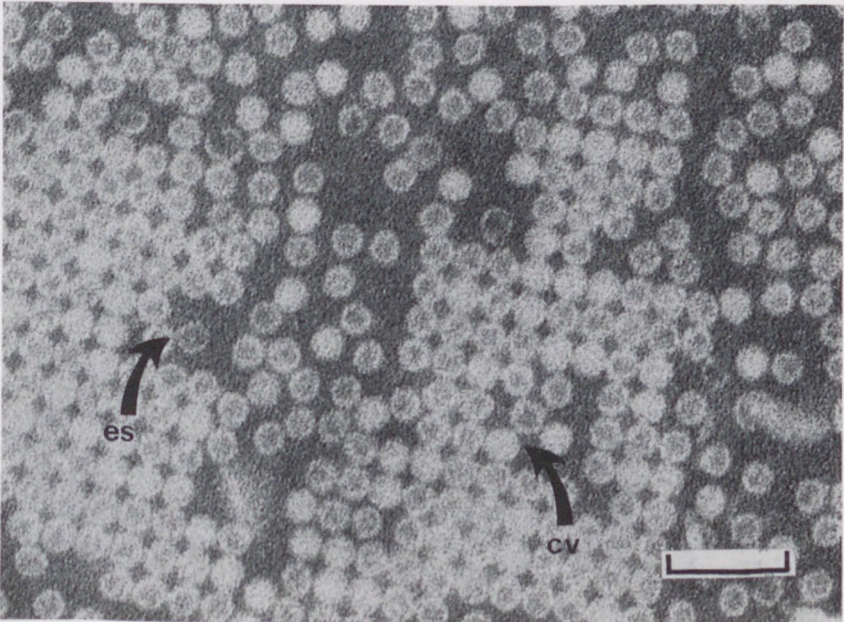
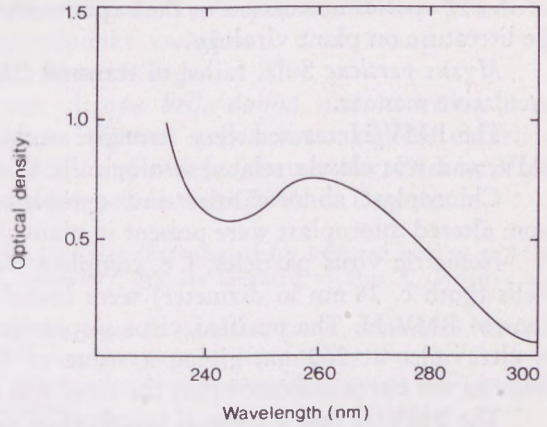


Fig. 7. Particles of partially purified H isolate of belladonna mottle virus under the electron microscope. cv, complete virions; es, virus protein empty shells. Bar represents 100 nm

Summary

In the course of investigations presented here, one virus isolate from *Atropa bella-donna* L. plants in Hungary was identified as belladonna mottle virus (BMV, cryptogram: R/1 : 2.0/37 : S/S : S/Cl). Reactions of test plants and host range of H isolate of BMV (BMV/H) were very similar to those of BMV isolates described previously. Forty-eight plant species were demonstrated as new experimental hosts, and 51 species as new resistant plants of BMV/H.

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