Isometric virus affecting bean plants in Lithuania

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Samples of common bean leaves with symptoms suggestive of virus infection were collected from private gardens in 1998. Two bean virus isolates were investigated by methods of test-plants and electron microscopy. This isometric virus infected mechanically inoculated bean plants which showed an exceptional local reaction on leaves, later followed by a severe systemic necrosis leading to the premature death of the plant. The properties of this agent were different from the other identificated viruses affecting bean plants in Lithuania. The agent could be ascribed to *bean southern mosaic virus* from the genus *Sobemovirus* on the basis of the narrow host range, severe symptom reactions, morphology of particles. The results of the virus purification and investigation of possible transmission by seeds are presented.

Key words: bean, virus diseases, viruses, identification

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important legume crops rich in protein. It is grown for green tender pods as well as for dry seeds which are used as vegetables. Virus diseases are responsible for heavy losses in yield and seed quality. Bean plants are affected by 12–14 viruses [1, 2]. According to symptom expression on the source plant and plant indicators, the virus morphology and the properties of viruses in plant sap of naturally affected bean plants two viruses – *bean yellow mosaic potyvirus and bean commom mosaic potyvirus* – were isolated and identified in Lithuania [3].

Field-grown beans showing different symptoms, plant stunting, brown spots, mottle pattern on distorted leaves were obtained in 1998. Analysis of the leaf extract by electron microscopy revealed isometric virus particles. The paper presents first data on the isometric virus as a causal agent of bean virus disease in Lithuania.

MATERIALS AND METHODS

Bean plants showing symptoms of virus disease were collected in private gardens of Vilnius and Širvintos regions. Two isolates from naturally infected bean plants were investigated and maintained in a greenhouse of the Plant Virus Laboratory. Test plants belonging to the following families were inoculated mechanically with sap from systemically infected bean leaves: *Aizoaceae* Rudolphi, *Amaranthaceae* Juss., *Asteraceae* Dum., *Chenopodiaceae* Vent., *Fabaceae* Lindl., *Solanaceae* Juss. The inoculum for mechanical inoculation was prepared by homogenizing infected leaves in an equal volume of distilled water or 0.1 M phosphate buffer, pH 7.0, containing 0.2% 2-mercaptoethanol or 0.01 M sodium diethyldithiocarbamate. Crude sap preparations were then inoculated onto a series of indicator plants (see Table). Inoculated test-plants exhibited the symptoms in 4–6 days. The plants were tested for symptomless infection by back-inoculation to *P. vulgaris.*

Virus particles from sap extracts of infected plants and purified virus preparations were visualized in preparations negatively stained with 2% uranyl acetate, using a JEM 100 S electron microscope [4, 5].

For investigation of virus transmission by seeds, healthy bean seedlings of cv. 'Bataaf' and 'Red Kidney' were mechanically inoculated by the virus. The seedlings were observed for symptom expression during a week. Mature seeds were collected from the beans showing typical symptoms. The seedlings of bean plants were grown in greenhouse. Fourty bean seedlings (20 seedlings of each cultivar) were examined for presence of virus infection by means of electron microscopy, and the development of symptoms was observed.

The virus isolates N 9809 and N 9810 were purified from infected bean leaves according to the modified method based on [6, 7]. Locally and systemically infected leaves were harvested 10–12 days after inoculation. Plant tissue was homogenized in cold 0.25 M phosphate buffer pH 7.2 with a stabilizing agent – 0.1% 2-mercaptoethanol (1:2 w/v) at 10000 g for 3 min and filtered through cheesecloth. The pH of tissue homogenate was adjusted to 6.5 with ascorbic acid. The extract was clarifited by ad-

Table. **Symptoms produced by bean virus isolates in different test plant species**

No	Plant indicators	Isolates and caused symptoms	
		9809 – mild	9810 – severe
1	*Amaranthus caudatus* L.	0	0
2	*A. paniculatus* L.	–	0
3	*Celosia argentea f. cristata* (L.) Kuntze	0	0
4	*Chenopodium ambrosioides* L.	(R LL)	(R LL)
5	*C. amaranticolor* Coste et Reyn	0	0
6	*C. capitatum* L.	0	–
7	*C. foetidum* Schrad.	0	(Chl LL)
8	*C. murale* L.	0	0
9	*C. quinoa* Willd.	(Chl LL)	(Chl LL)
10	*C. urbicum* L.	0	0
11	*Datura stramonium* L.	0	0
12	*Glycine max* (L.) Merr	0	(S: VC)
13	*Gomphrena globosa* L.	0	0
14	*Lupinus albus* L.	0	0
15	*L. angustifolius* L.	–	0
16	*Nicandra physalodes* (L.) Gaertn.	0	0
17	*Nicotiana affinis* Moor.	–	0
18	*N. debneyi* Domin.	0	0
19	*N. glutinosa* L.	0	0
20	*N. langsdorfii* Weinm	–	0
21	*N. rustica* L.	0	0
22	*N. sylvestris* Speg. and Comes	0	0
23	*N. tabacum* L. cv. Xanthi	0	0
	cv. Samsun	0	–
24	*Phaseolus vulgaris* L. cv. Bataaf	NLL; S:Mo, LCu, St, TN	NLL; S:VC, LCu, St, TN, D
	cv. Red Kidney	NLL; S:VC, St, TN	NLL; S:VC, Mo, St, TN, D
	cv. Zlota saxa	NLL; S:Mo, LCu, St	NLL; S:Mo, LCu, St, TN, D
	cv. Motolskaja belaja	NLL; S:VC, LCu, St,	NLL; S:Chl Mo, LCu, St,
25	*Pisum sativum* L. cv. Žalsviai	0	0
	cv. Rainiai	0	0
26	*Tetragonia expansa* Murr.	0	0
27	*Verbesina encelioides* Benth et Hook	0	0
28	*Vicia faba* L. cv. Aušra	0	0
	cv. Windsor	0	–
29	*Vigna sinensis* (L.) Savi ex Hassk.	(Br LL)	(Br LL)
30	*Zinnia elegans* Jacq	0	0

Abbrevations: LL – local lesions, N – necrotic, Chl – chlorotic, Br – brown, R – red, S – systemic reaction, Mo – mottling, LCu – leaf curling, St – stunting, TN – top necrosis, VC – vein clearing, D – decline of plants, ( ) – questionable symptoms, 0 – plant not reacted, – – plant not tested.

ding 10% (w/v) butanol and chloroform mixture 1:1 and stirred at room temperature for 30 min and then centrifuged at 8000 g for 10 min. Virus particles were precipitated by adding 8% (w/v) polyethylene glycol Mw 6000 and 1.5% sodium chloride and by two cycles of differential centrifugation. Further purification was carried out by high-speed (28000 g during 3 h) centrifugation through 30% (w/v) sucrose cushion made in the same buffer. The pellet was resuspended overnight at 4 °C in a small volume of 0.025 M phosphate buffer pH 7.2 and clarified on a low-speed K-24 centrifuge. Final purification was accomplished by sedimentation through a 25% sucrose cushion. The virus concentration and yield were estimated by electron microscopy and spectrophotometrically assuming $A^{0.1\%}_{260nm} = 5.85$, which is within the range reported for *Sobemoviruses* [6, 8].

RESULTS AND DISCUSSION

Naturally infected bean plants showed severe mottling or chlorosis and malformation of young leaves. Differential test plant species were mechanically in-

oculated with a crude sap of naturally infected beans. The results of host range test and symptomatology are summarized in Table. Results of these tests showed that two isolates of virus locally and sometimes systemically infected only one of about 30 test species of plant indicators. Four bean cultivars tested in this experiment were susceptible to this virus. The virus always induced a very characteristic local response (dark brown blotched spots) on inoculated leaves of bean cv. 'Bataaf', 'Red Kidney', 'Motolskaja belaja', 'Zlota saxa'. Sometimes these cultivars showed symptoms consisting in necrotic local lesions on inoculated leaves, followed by systemic leaf curling and by stunting of bean plants. The necrotic lesions were small brown spots or flecks 1 to 3 mm in diameter (Fig. 1). Necrotic lesions on beans cv. 'Bataaf' were larger and more diffuse than on leaves of other bean cultivars (Fig. 2). The virus isolates sometimes caused a systemic reaction on the youngest leaves: vein clearing, mild green mottling or mild chlorosis. The size of leaves and of a whole plant are often reduced. The inoculated beans very often induced a drastic necrotic reaction, which was followed by the stunting, browning, wilting and death of beans. According to the severity of symptoms produced on beans, our isolates were considered as mild and severe types: 9809 – mild and 9810 – severe. The reaction of other test plants to a test agent was questionable. Both virus isolates produced pinpoint chlorotic, red or brown local lesions on *Chenopodium ambrosioides* L., *C. foetidum* Schrad. and *C. quinoa* Willd. in about a week after inoculation. The local lesions tended to enlarge irregularly. The virus isolate 9810 (severe) produced systemic mild vein clearing on *Glycine max* (L.) Merr. cv. 'Black eye'. However, the virus was not detected in this plant indicator by the method of electron microscopy. In our experiments, cowpea (*Vigna sinensis* (L.) Savi ex Hassk.) sometimes showed small brown local lesions or mild chlorosis on inoculated leaves. The virus infection, however, from *V. sinensis* was not mechanically transmitted to healthy cowpea and bean plants. The observed symptoms were induced not by virus infection. This virus propety allows to admit that it could be the bean strain of virus that did not infect cowpea plants [9]. Many of the indicator plants did not react to any of the virus isolates (see Table). These results of narrow host range and particular symptoms on beans of the study virus from beans most closely correspond to the properties of *bean southern mosaic sobemovirus* (BSMV) [1, 7, 10].

Isometric particles were readily visualized by the electron microscope in crude sap preparations from naturally infected bean samples or from inoculated bean plants (Fig. 3). Bean is affected by several isometric viruses: *bean leaf roll luteovirus, bean mild mosaic carmovirus, bean pod mottle comovirus, bean yellow vein banding umbravirus* and others, but these viruses have wide experimental host ranges [1, 2].

Fig. 1. Small necrotic local lesions on *P. vulgaris* cv. Red Kidney leaves after inoculation

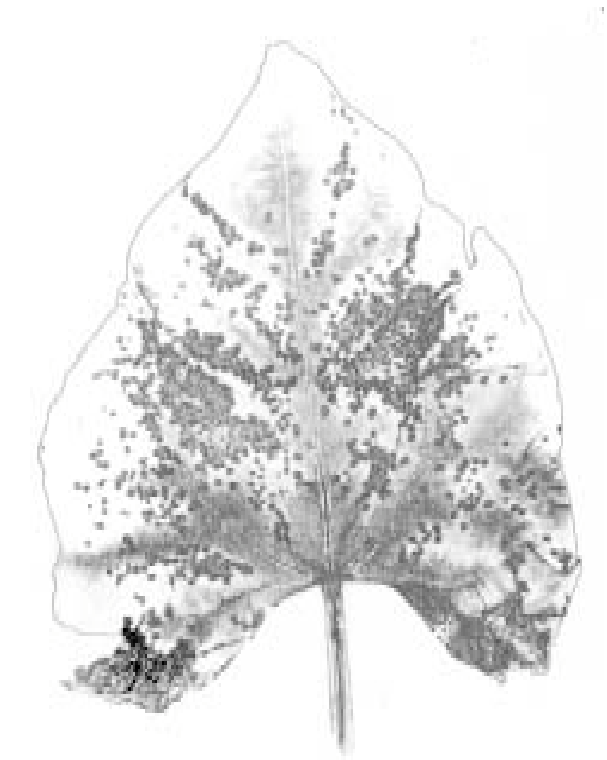

Fig. 2. Severe diffuse necrotic local lesions on *P. vulgaris* cv. Bataaf leaves after inoculation

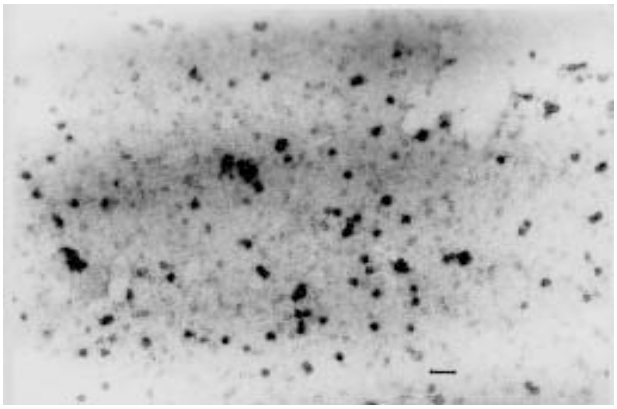

Fig. 3. Isometric particles in preparations from naturally infected or inoculated bean plants. Bar marker represents 100 nm

In our assays, the virus was not transmissible by seeds of beans cv. 'Bataaf' and 'Red Kidney', although BSMV has been reported to be seed-borne in 3–5% of bean and in 5–40% of cowpea [2, 10, 11, 12]. Seedlings grown from seeds collected from virus-infected plants did not express symptoms of virus disease, and virus particles were not found by electron microscopy. BSMV is transmitted by beetles *Ceratoma trifurcata* in North America and *Madurasia obscurella* in India [1, 2, 13].

The ultraviolet spectrum of partially purified preparations was typical of nucleoprotein, with maximum absorbance at 260 nm and minimum at 240 nm. The ratio of absorbance at 260/280 was 1.09–1.22. The yield of purified virus was estimated to about 3.2 4.9 mg/100 g fresh bean leaves. Electron micrograph of the purified virus revealed isometric particles about 30 nm in diameter, as have been reported by other investigators [2, 6, 14].

Based on the results of our experiments and literature data, this agent preliminarily could be attributed to BSMV from the genus *Sobemovirus*. BSMV occurs in the warm temperate regions of Europe, but its presence is also possible in other regions of Europe [2, 15].

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PUPELES PAŽEIDŽIANTIS IZOMETRINIS VIRUSAS LIETUVOJE

S a n t r a u k a

Daržinių pupelių su virusinės infekcijos simptomais pavyzdžiai buvo aptikti privačiuose daržuose 1998 m. Išskirti du viruso izoliatai buvo tiriami augalų indikatorių ir elektroninės mikroskopijos metodais. Lapų margumo, nekrotinio dėmėtumo ir žemaūgės sukėlėjas izometrinis virusas mechaninės inokuliacijos bandymuose užkrėtė 4 veislių pupeles, sukeldamas lapuose vietinę reakciją, vėliau pereinančią į sisteminę nekrozę ir lemiančią ankstyvą augalo žūtį. Tiriamasis virusas biologinėmis savybėmis skiriasi nuo kitų Lietuvoje identifikuotų pupeles pažeidžiančių virusų. Pagal ypač siaurą eksperimentiškai pažeidžiamų augalų spektrą, specifinius reakcijos simptomus juose, virionų morfologiją ir bandymo perduoti virusą su sėkla rezultatus tiriamasis izometrinis virusas iš pupelių lapų galėtų būti preliminariai pavadintas pupelių pietinės mozaikos sobemovirusu (*Bean southern mosaic sobemovirus*). Straipsnyje pateikiama viruso izoliatų gryninimo eiga ir rezultatai.