CHARACTERIZATION OF THE ALFALFA MOSAIC VIRUS STRAIN T6

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Summary. – A strain T6 of alfalfa mosaic virus (AlMV) was characterized. It was isolated from field grown lucerne. Purified virus preparations contained four types of particles, B, M, Tb and Ta, containing separately encapsidated ssRNAs 1 to 4. The strain T6 was able to infect 40 different plant species of 9 families, and to develop a systemic infection in most of them. The symptomatology on bean and the RNA mobility of the AlMV strains T6 and 425 were compared. The classical cross-protection experiments on bean have shown that plants inoculated with strain 425 did not develop symptoms of the challenge strain T6.

Key words: alfalfa mosaic virus; host range; symptomatology; cross-protection; RNA

Introduction

AlMV is a world-wide distributed plant virus with unique morphology. The virus is found mainly in legumes, but also in other dicotyledonous plants including some woody hosts. The symptoms of infection are diverse (e.g., mosaic, mottling, necrotic and chlorotic local lesions, systemic necrosis etc.), and depend strongly on the virus strain, host variety, stage of growth and experimental conditions (Jaspars and Bos, 1980). A symptomless infection occurs quite often.

The genome of AlMV consists of three ssRNAs. RNA 1 and 2 encode the replicase proteins P1 and P2 (Nassuth and Bol, 1983), and bicistronic RNA 3 encodes the movement protein (P3) and the coat protein (CP) (Cornelissen and Bol, 1984). CP is translated from subgenomic RNA 4. The four RNAs (1-4) or genomic RNAs complexed with a small amount of CP are essential for the infection of plants (Bol *et al.*, 1971).

In this paper we present characterization of the AlMV strain designated T6 (Gallo, 1977). Some properties of this strain are compared with the AlMV strain 425 (Hagedorn and Hanson, 1963).

Materials and Methods

AlMV T6 isolation was done by a repeated single lesion passaging on *Phaseolus vulgaris* L.cv. Bountiful of an isolate from *Medicago sativa* L. grown in a field near the village Troubsko (Czech Republic). The virus strain was maintained by passaging on *Pisum sativum* L.cv. Juran.

Virus multiplication and purification. The strains T6 and 425 were propagated on *P.sativum* cv. Juran grown in greenhouse at 22 °C and 16 hrs light period. After 12 – 14 days the infected plant material was homogenized in 0.1 mol/l phosphate buffer pH 7.0, and the extract was rehomogenized with chloroform. The virus was precipitated from the aqueous phase with 8% (w/v) polyethylene glycol 6,000 in the presence of 0.3 mol/l NaCl. The pellet was resuspended in 0.1 mol/l phosphate buffer and clarified by low speed centrifugation. For further purification we used a centrifugation on a 20% sucrose cushion and a 15 – 40% sucrose gradient centrifugation. The viral fraction from the gradient was dialyzed against the extraction buffer, pelleted by high speed centrifugation (150,000 x g, 90 mins), and finally resuspended in 10 mmol/l phosphate buffer pH 7.0.

Electron microscopy. Purified virus was loaded onto grids, fixed by formaldehyde, and stained with phosphotungstic acid (Hull, 1969). The grids were examined in a Philips EM 300 electron microscope operating at 80 kV.

Host range test. The infectivity of the strain T6 was tested by mechanical inoculation on 40 different plant species of 9 families. The concentration of the virus in the inoculum was 0.1 mg/ml. The experiments were repeated 3 times in various seasons, and the effect of infection was evaluated 9 – 28 days after inoculation.

RNA isolation. Disodium ethylenediamine tetraacetate (EDTA) was added to the purified virus preparation to final concentration of 10 mmol/l, and RNA was isolated by repeated phenol and phenol-chloroform extractions. After ethanol precipitation and washing in 75% ethanol RNA was dissolved in TE_5 buffer (10 mmol/l Tris-HCl pH 8.0 and 5 mmol/l EDTA).

Abbreviations: AlMV = alfalfa mosaic virus; CP = coat protein; EDTA = ethylenediamine tetraacetate

Agarose gel electrophoresis. RNA samples were denatured in the presence of 8% formaldehyde (65 °C, 10 mins) and electrophoresed (3V/cm) in non-denaturating agarose (1%) gel. Gels were stained in ethidium bromide or toluidine blue solutions.

RNA infectivity assay. Leaves of Bountiful bean were inoculated with total RNA (5 μg) and with various mixtures of single RNAs. The inoculated plants were placed in a greenhouse and grown under conditions described above.

Results

The AlMV strain T6 was isolated by Gallo in 1974 from cultivated lucerne carrying the symptoms of systemic infection (chlorotic spots and leaf distortion). The electron micrograph (Fig. 1) shows a purified virus preparation consisting of four components: B (bottom), M (middle), Tb (top b), and Ta (top a). There were also smaller spherical particles visible on the micrograph.

T6 is severely pathogenic strain. Under experimental conditions used, it infected 40 different plant species of 9 families, and caused systemic infection on all assayed plants with the exception of *Calendula officinalis* L., *Antirrhinum majus* L., and *Dolichos lablab* L. On mechanically inoculated leaves of some plants, e.g., *Cucumis melo* L., *Medicago sativa* L., and/or *Trifolium pratense* L., there did not arise the primary symptoms, but only the symptoms of a systemic infection. Some plants as *Lactuca sativa* L. and/or *Tetragonia expansa* Murr. were infected latently. The latent infection was proved by reinoculation of a sap from the apical leaves on pea or bean. The symptomatology of AlMV strain T6 infection on selected hosts is presented in Table 1. To demonstrate the strain specificity, the effect of T6 and 425 infections on cv. Bountiful was compared. The strain 425 produced minute local necrotic lesions on inoculated leaves after 2 – 4 days; they were of a constant size during further cultivation (Fig. 2B). No systemic symptoms were observed. On the other hand, the strain T6 caused in the same concentration "growing" lesions (Fig. 2A) and systemic infection with apical symptoms (Fig. 3). Since both strains have capabilities to produce different symptoms on Bountiful bean, a classical cross-protection tests were done. The bean leaves were inoculated with the strain 425. After 18 hrs (no local necrotic lesions were developed) and 2 days (local necrotic lesions were visible), the leaves were superinfected with the

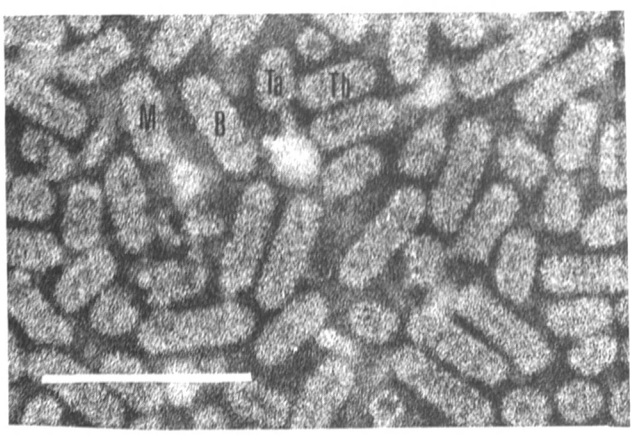

Fig. 1
Electron micrograph of AlMV strain T6 showing the four components – B,M,Tb and Ta
Bar = 100 nm.

Table 1. Symptomatology of AlMV strain T6 infection on selected hosts

Host plant	Symptoms	
	inoculated leaves	systemic infection
Gomphrena globosa	LNL, CLS	CLS, VN
Calendula officinalis	CLS	0
Lactuca sativa	CLS	La
Chenopodium amaranticolor	CLS	CLS, LD
Cucumis sativus	LNL	M
Pisum sativum	LNL	VN, AN, SN
Trifolium pratense	La	VN, M
Vicia faba	LNL	SN, AN
Tetragonia expansa	CLS	La
Antirrhinum majus	LNL	0
Nicotiana glutinosa	LCL	CLS, LD
Petunia hybrida	CLS	M
Lycopersicon esculentum	LNL	LD, AN
Nicandra physaloides	LNL	CLS, LD

AN = apical necrosis; CLS = chlorotic leaf spot; La = latent infection; LCL = local chlorotic lesion; LD = leaf deformation; LNL = local necrotic lesion; M = mosaic; SN = stem necrosis; VN = veinlet necrosis; 0 = no systemic infection.

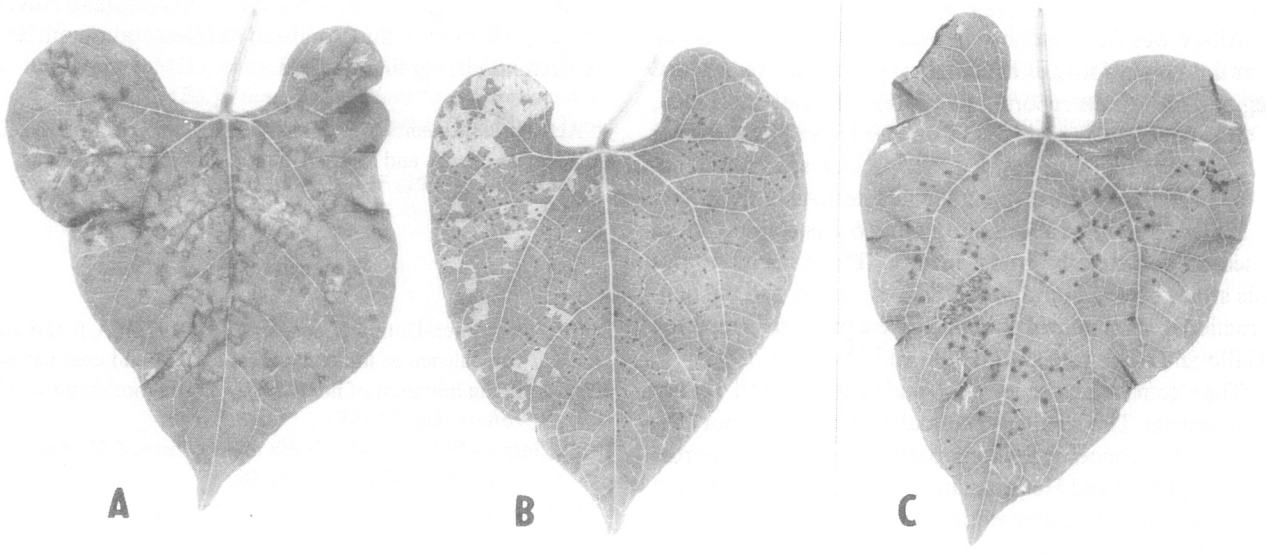

Fig. 2
Symptoms of AlMV infection on cv. Bountiful leaves 9 days p.i.
A – strain T6, B – strain 425, C – T6 RNA.

strain T6 (challenge virus). In both cases the growing lesions and symptoms of a systemic infection did not develop. In an experiment performed conversely (the plants were challenged with the strain 425), both types of lesions on inoculated leaves and symptoms of systemic infection were present. In a mixed infection both types of lesions appeared, but after a prolonged cultivation the plants carried no symptoms of a systemic infection.

The genome analysis in non-denaturating agarose gel electrophoresis demonstrated four RNAs (Fig. 4). There were small differences in the mobility of RNA 2 and 3 between the strains T6 and 425. Only a mixture of all RNAs (1-4) was infectious, but the characteristic lesions of the strain T6 appeared later, namely 9 – 11 days after RNA inoculation (Fig. 2C).

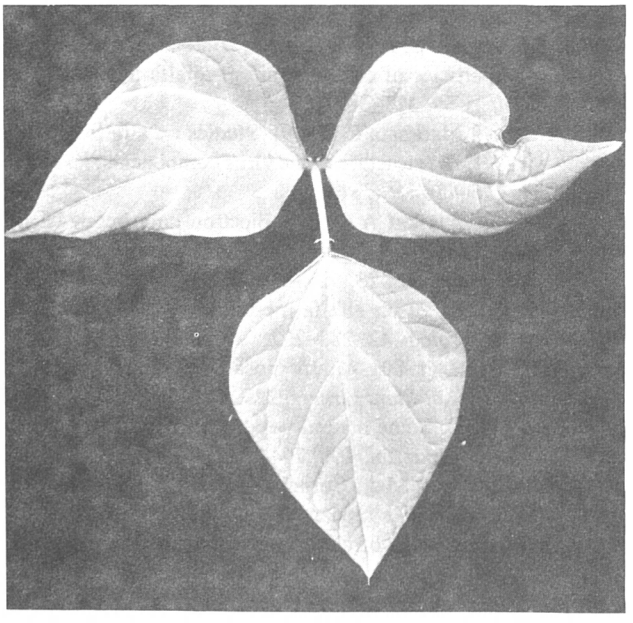

Fig. 3
Symptom of a systemic infection with AlMV strain T6 on apical bean leaf

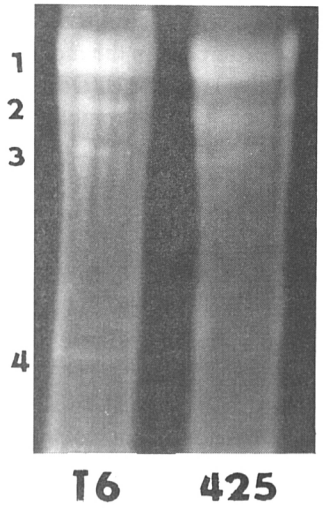

Fig. 4
Agarose gel electrophoresis of RNAs of ALMV strains T6 and 425
1 – RNA 1, 2 = RNA 2, 3 = RNA 3, 4 = RNA 4.

Discussion

AlMV was first described by Weimer (1931, 1934). Since that time many isolates and strains from various hosts and regions have been reported (Van Regenmortel and Pinck, 1981). AlMV was also found on the territory of former Czechoslovakia (Musil *et al.*, 1966; Kvíčala, 1975; Gallo, 1977), but the isolates were not characterized in detail.

As we mentioned above, the strain T6 was obtained from diseased comercially grown lucerne. The transmission of this strain by *Nicandra physaloides* L. seeds, and the construction of monoclonal antibodies were previously reported (Gallo and Čiampor, 1977; Gallo and Matisová, 1993).

The electron microscopy of T6 purificates showed 4 major components. The smaller spherical particles were not analysed. What concerns the long particles of AlMV reported by Hull (1970) and Heijtink and Jaspars (1974) we did not find them in our T6 preparations.

That AlMV has a wide range of hosts (Jaspars and Bos, 1980) was confirmed also by the investigation of the T6 host range. According to its symptomatology on cv. Bountiful, the strain T6 differs from the strain 425. The former overcomes the host defense mechanism(s) and spreads via cell to cell ("growing" lesions) and long distance movement. The expression of T6 symptoms was suppressed if cv. Bountiful plants were protected with the strain 425, and challenged with the strain T6. A partial inhibition of T6 symptoms (no systemic symptoms) was observed after infection of cv. Bountiful bean with a mixture of both strains. As we analyzed this phenomenon only on the level of symptomatology, further experiments are necessary.

Another difference between the strains T6 and 425 was in the mobility of RNA 2 and 3. The position of RNA 3 and 4 was verified by Northern blot analysis using a cDNA probe coding for coat protein of the strain 425 (data not shown). Analogous difference in the mobility has been described for AlMV strains L, S and B (Dore *et al.*, 1989). A comparison of sequences of bicistronic RNA 3 of various AlMV strains showed significant dissimilarities in the 5'-leader sequences (repeated sequences, number of nucleotides) and in the coding region (nucleotide and amino acid changes) (Langereis *et al.*, 1986; Neelman *et al.*, 1991; Van der Vossen *et al.*, 1993). Although the sequences of T6 RNAs are not yet known, the mobilities of RNAs and biological properties of T6 indicate the presence of T6-specific sequences.

The infection of bean with purified T6 RNAs 1-4 produced symptoms of a complete virus infection. Missing coat protein in early steps of infection (Neeleman *et al.*, 1991; Van der Vossen *et al.*, 1994) and/or low concentration of viral RNA might cause a delayed development of symptoms after RNA inoculation.

Biological and structural properties of the T6 isolate characterized in this paper show that it is a new strain of AlMV. Further properties of this strain and its interactions with host are currently being investigated.

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