Chinese Industry and Technology of Eco-environment
2026, VOL. 7, NO. 1, 50-52
DOI: 10.47297/wspciateWSP2633-772X10.20260701

Infectivity Characteristics of Alfalfa Mosaic Virus Infectious Clone

Yushu Huang

Faculty of Science, University of Copenhagen, Copenhagen, 2100, Denmark

ABSTRACT

Alfalfa mosaic virus (AMV) is a major constraint on alfalfa production in China. Infectious cDNA clones provide a stable system for viral preservation and functional studies. Here, an AMV infectious clone was used to assess pathogenicity and its impact on host growth. Mechanical inoculation showed that Nicotiana benthamiana, but not Medicago sativa, developed typical mosaic symptoms, with a 75% infection rate and a disease index of 33.89 at 10 days post-inoculation. Infected plants exhibited reduced fresh weight and increased dry weight, indicating tissue dehydration, while both total nitrogen and crude protein levels showed no significant change. These results demonstrate that the AMV infectious clone is pathogenic to Nicotiana benthamiana and provides an effective tool for future studies on AMV–host interactions and resistance breeding in alfalfa.

KEYWORDS

Alfalfa mosaic virus, Infectious clones; Nicotiana benthamiana; Medicago sativa; Pathogenicity; Molecular identification

Alfalfa (Medicago sativa L.) is a major forage crop in China, valued for its high yield, protein content, and palatability. However, viral diseases particularly Alfalfa mosaic virus (AMV), have increasingly limited production. AMV occurs widely in major alfalfa-producing regions of China, with detection rates reaching up to 100%, causing dwarfing, chlorosis, and reduced biomass and protein content[1]. With a host range spanning more than 700 species across over 70 families[2] and transmission primarily via aphids and contaminated seeds[3], AMV is difficult to control, and its pathogenic mechanisms remain poorly understood[4].

Infectious cDNA clones provide a powerful tool for preserving viral isolates and enabling controlled inoculation, facilitating studies of viral gene function5 and host resistance6. However, infectious clone research in alfalfa remains limited, and no AMV clone has been evaluated for pathogenicity in host plants. This study therefore investigates the pathogenic characteristics of an AMV infectious clone and its effects on host physiology, providing a basis for understanding AMV–host interactions and supporting resistance breeding in alfalfa.

To evaluate AMV pathogenicity, an infectious clone was introduced into Agrobacterium tumefaciens and inoculated into Nicotiana benthamiana and Medicago sativa. Transformants were selected on Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, 15 g/L agar) supplemented with kanamycin and rifampicin (50 μg/mL). A single colony was cultured in liquid LB at 28 °C and 180 rpm, verified by PCR, and adjusted to OD_{600} = 1.0 in MMA buffer (10 mM 2-(N-morpholino) ethanesulfonic acid, 10 mM $MgCl_2$, 100 μM acetosyringone). The bacterial suspension was infiltrated into the abaxial surface of two leaves per plant, and empty- vector plants were used as controls.

Disease symptoms were recorded for 10 days, and the pathogenicity was evaluated based on disease incidence and disease index.

Disease incidence was calculated as:

$$Disease\ incidence = \frac{Number\ of\ infected\ plants}{Total\ incovulated\ plants} \times 100\%$$

Disease severity was scored according to a 0–9 scale[7] and the disease index was determined using:

$$Disease\ index = \frac{\sum \left(Disease\ grade \times Number\ of\ plants \right)}{Total\ plants \times 9} \times 100$$

Total RNA was extracted from inoculated and systemic leaves using TRIzol, and cDNA was synthesized with the TRUEscript kit. PCR amplification employed AMV RNA 1– RNA 3 specific primers8 under the following conditions: 94℃ for 2 min; 30 cycles of 94℃ for 30 s, 55℃ for 30s, 72℃ for 30s; and a final extension at 72℃ for 2 min. PCR products were analyzed by agarose gel electrophoresis and were sent to Beijing Ruibo Xingke Biotechnology Co., Ltd. for sequencing. After obtaining the sequencing results, BLAST analysis of the sequences were performed on NCBI.

At the late infection stage, plants were harvested and fresh weight measured. Samples were blanched at 105℃ for 30 min, oven-dried at 65℃ for 48 h, and dry weight was recorded to calculate dry-to-fresh weight ratios and water content. Total nitrogen (TN) was determined, and crude protein (CP) content was calculated using:

$$x = \omega \times 6.25$$

Where x is the CP content (g/100 g) and ω is the nitrogen content (g/100 g).

Following inoculation with the AMV infectious clone, Nicotiana benthamiana developed typical mosaic symptoms that

* **Corresponding Author:** Yushu Huang, cbx387@alumni.ku.dk

progressed over time, whereas no symptoms were observed in the control plants (See Figure 1).

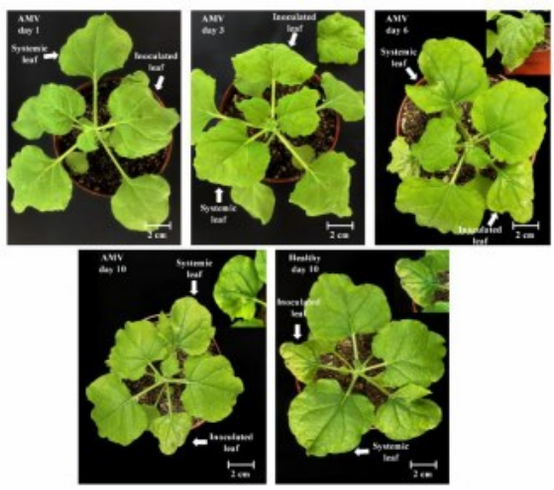

Figure 1 Symptoms of Nicotiana benthamiana plants infected by AMV

Disease incidence and disease index reached 75% and 33.89 by 10 days post-inoculation (See Figure 2), indicating stable pathogenicity of the clone. AMV RNA 1–RNA 3 were detected in both inoculated and systemic leaves by RT-PCR (See Figure 3), confirming successful viral replication and systemic movement.

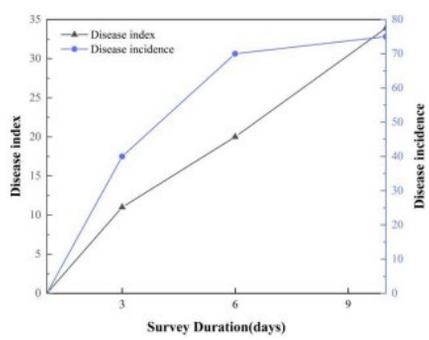

Figure 2 Disease grade statistic and incidence rate of Nicotiana benthamiana plants infected by AMV

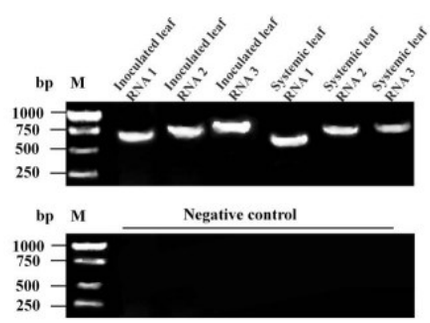

Figure 3 RT-PCR detection of AMV RNA gene in the leaves of Nicotiana benthamiana plants

To evaluate physiological effects, biomass was measured at the late infection stage. Infected plants showed significantly reduced fresh weight but increased dry weight and decreased water content ($P < 0.05$; See Table 1), suggesting that AMV disrupts host water balance. In contrast, total nitrogen and crude protein contents did not differ significantly between treatments (See Table 2), indicating that AMV affects water metabolism might be earlier than nitrogen metabolism.

Table 1 Biomass statistics of Nicotiana benthamiana plants infected by AMV

Group	Treatment	Fresh weight/g	Dry weight/g	Water content/%	Dry- fresh ratio
1	Control	3.67 ±0.41	0.40 ±0.06	88.85 ±1.76	0.11 ±0.01
	Treatment	1.69 ±0.19*	0.76 ±0.08*	54.78 ±0. 14*	0.45 ±0.00*
2	Control	4.63 ±0.08	0.48 ±0.03	89.72 ±0.40	0.10 ±0.00
	Treatment	3.06 ±0.23*	0.93 ±0.13*	68.62 ±6.86*	0.31 ±0.07*
3	Control	6.60 ±0.69	0.93 ±0.09	85.94 ±0.15	0.14 ±0.00
	Treatment	3.99 ±0.35*	1.00 ±0. 11	73.97 ±4.61	0.26 ±0.05

The values in the table are average value ± standard deviation and * represent significance level ($P<0.05$).

Table 2 TN and CP content statistics of Nicotiana benthamiana plants infected by AMV

Group	Treatment	ω(g/100g)	Average value± standard deviation	X(g/100g)	Average value± standard error
1		2.89		18.06	
2	Control	211	2.49±0.225	13.19	15.58±1.406
3		2.48		15.5	
1		2.58		16.13	
2	Treatment	2.36	2.54±0.097	14.75	15.90±0.606
3		2.69		16.81	

In the table, ω (g/100 g) represents the total nitrogen content of the plant, and X (g/100 g) denotes the crude protein content of the plant.

In Medicago sativa, no visible symptoms or AMV-specific RT-PCR bands were detected (See Figure 4), suggesting thatthe infectious clone failed to establish systemic infection in alfalfa, likely due to host resistance or insufficient viral accumulation.

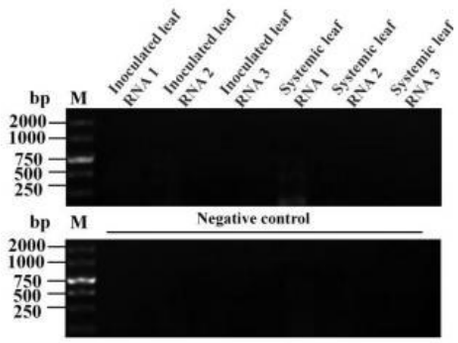

Figure 4 RT-PCR detection of AMV RNA gene in the leaves of Medicago sativa plants

Collectively, the AMV infectious clone causes typical systemic infection and physiological disruption in Nicotiana benthamiana, but fails to infect alfalfa.

The results of this study demonstrate that the AMV infectious clone can establish systemic infection in Nicotiana benthamiana, as evidenced by typical mosaic symptoms, increasing disease index, and positive RT-PCR detection. These findings indicate that the clone exhibits pathogenic characteristics comparable to those of field AMV isolates[2]. Infected plants showed significantly reduced fresh weight and increased dry matter, suggesting that AMV disrupts water homeostasis and induces tissue dehydration, consistent with previous reports that AMV can alter hormone balance and osmotic regulation in host plants[9]. In contrast, nitrogen content and crude protein contents were not significantly affected, implying that AMV infection initially impacts water metabolism rather than nitrogen metabolism, which aligns with observations that nitrogen-related effects appear at later infection stages[10]. Meanwhile, no AMV-specific amplification bands were detected in Medicago sativa, indicating that the infectious clone failed to establish systemic infection in alfalfa. This result may be related to host resistance, insufficient viral accumulation, or limitations in the inoculation method. Together, these findings not only verify the pathogenicity of the AMV infectious clone in a susceptible host, but also highlight distinct host responses that warrant further investigation into AMV-host interactions.

References

[1] Zhou Q, Liang Q, Han L. Symptoms and pathogen detection of alfalfa virus disease[J]. Pratacultural Sci, 2016, 33: 1297-1305.

[2] GUO Z, FENG C, ZHANG J, et al. Field resistance to alfalfa mosaic virus among 30 alfalfa varieties[J]. Acta Prataculturae Sinica, 2019, 28(4): 157.

[3] Yang Yucui, Ni Ying, Zhao Jinxia, et al. Occurrence patterns and control measures of alfalfa aphids in Shizuishan City, Ningxia [J]. Jilin Agriculture, 2012, (08): 72.

[4] Li Meirong, Sun Yuan, Lu Jianlin, et al. Research progress on alfalfa mosaic virus [J]. Guizhou Agricultural Sciences, 2019, 47(04):69-73.

[5] Tang, Muning. Study on pathogenicity differentiation of common bean mosaic virus (BCMV) and construction of infectious and chimeric infectious clones [D]. Shanxi Agricultural University, 2023.

[6] Gao, Guofeng. Study on the molecular mechanism of NbLTP2, a plant ABA-responsive gene, enhancing antiviral activity [D]. Shandong Agricultural University, 2023.

[7] You Tanggui, Duan Yanru, Zhang Yan, et al. Control effects of four virus inhibitors on tomato spotted wilt virus and potato virus Y in Zhaotong tobacco [J]. Chinese Agricultural Science Bulletin, 2021, 37(36):135-141.

[8] Zhang, Xinwei. Molecular identification and detection of pathogens of alfalfa witches' broom and mosaic virus in Xinjiang [D]. Xinjiang Agricultural University, 2016.

[9] Tian Long, Liang Qiaolan, Wei Liexin, et al. Effects of AMV and WCMV co-infection on four endogenous hormones in Nicotiana benthamiana [J]. Chinese Journal of Grassland Science, 2023, 45(11):99-108.

[10] An D. Acetylation Regulation of NbGOGAT in Nitrogen Metabolism during TSWV Infection in Tobacco; Master's Thesis, Chinese Academy of Agricultural Sciences, 2023; p 53.