

インゲンマメモザイクウイルスのアズキモザイク系統ゲノムの3'末端領域の塩基配列と,インゲンマメモザイクウイルスグループ内での系統学的関係

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Nucleotide Sequence of the 3'-terminal Region of the Genome of the Azuki Bean Mosaic Strain of the Bean Common Mosaic Virus and Its Phylogenetic Relationship to Viruses in the Bean Common Mosaic Virus Group*

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Key words: bean common mosaic virus, azuki bean mosaic strain, nucleotide sequence, phylogenetic analysis.

A nucleotide sequence analysis of azuki bean mosaic virus (AzMV) showed that its coat protein (CP) gene has nearly 90% amino acid sequence identity with those of bean common mosaic virus (BCMV) strains^{1,2)}. In this paper, we propose that AzMV should be treated as the azuki bean mosaic strain of BCMV (BCMV-AzM). We determined the 3'-terminal sequence of an isolate of BCMV-AzM from Hokkaido, Japan (BCMV-AzM-JH), and compared the CP gene and 3' non-coding region of this isolate with those of other viruses in the BCMV subgroup. In order to elucidate the evolutionary relationships among viruses in the BCMV subgroup, we phylogenetically analyzed their amino acid and nucleotide sequences for the CP gene and the 3' non-coding region.

BCMV-AzM-JH was obtained from an azuki bean (*Phaseolus angularis* cv. Maruba) growing in a field plot at Hokkaido University in Sapporo, Japan. The virus, which induced a typical mosaic pattern, was mechanically transmitted to other azuki beans (*P. angularis* cv. Erimo Syozu) and maintained in a greenhouse. To test the transmissibility of the virus in seeds, azuki bean seeds were collected from two cultivars of BCMV-AzM-JH-infected plants, Erimo Syozu and Maruba, and grown in a greenhouse. Eight of thirty-one Erimo Syozu seedlings and seven of thirty-two Maruba seedlings showed typical mild mosaic symptoms, which is the biological response to AzMV^{8,9)}.

To identify its pathotype⁴⁾, BCMV-AzM-JH was used to mechanically inoculate the following *P. vulgaris* cultivars: Sutter Pink, Greenleaf C, Great Northern 31, Great Northern 123, Red Mexican, Pinto 114 and Black Turtle Soup 1. Sutter Pink was the only cultivar infect-

ed, which suggests that the isolate has the same host range as BCMV strains in pathogroup I⁴⁾.

BCMV-AzM-JH was partially purified from *Nicotiana benthamiana* and its genomic RNA was extracted. cDNA was synthesized with an oligo d(T) primer using a cDNA synthesis system (Amersham) and cloned into *Sma*I-digested pUC119. Fourteen overlapping cDNA clones containing the 3' terminal region were obtained and all of them were sequenced in both directions using a Thermo Sequenase fluorescent-labeled primer cycle sequencing kit (Amersham).

The 3' terminal 2167 nucleotide sequence of BCMV-AzM-JH RNA was determined including most of the NIb gene (1051 nt), the CP gene (864 nt), and the 3' non-coding region (252 nt). The dipeptide QS was selected as the putative cleavage site for the CP of BCMV-AzM-JH, because cleavage of potyviral polyproteins occurs mainly within the dipeptide Q/A or Q/S⁵⁾. The CP consists of 287 amino acids, and its predicted size is 32.3 kDa. Although the CPs of BCMV-AzM-W and dendrobium mosaic virus (DeMV) contain 285 amino acids, the BCMV-AzM-JH CP is the same length as the CPs of many BCMV strains (287 amino acids).

When the CP amino acid sequence was compared with those of BCMV-AzM-W and DeMV, the identity levels were 93.9% (BCMV-AzM-JH and BCMV-AzM-W), 90.5% (BCMV-AzM-JH and DeMV) and 93.0% (BCMV-AzM-W and DeMV). The CP amino acid sequences of BCMV-AzM-JH, BCMV-AzM-W, DeMV, BCMV-US4, BCMV-NL2, and peanut stripe virus (PStV)-OK were aligned (Fig. 1). The N-terminal region of the CP (amino acids 1-50), which is the most variable part of the poty-

* The nucleotide sequence data will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession number AB012663.

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	10	20	30	40	50	60	70	80	90																																	
BCMV-AzM-JH	SGTNQ	SSTPV	VDA	GDA	GDK	KKES	NK	KG	PES	KEG	SG	DS	SR	GA	ENS	SM	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	MP	VK	GN	MT	LN	DH	LL							
BCMV-AzM-W	SGINQ	P--	PE	VDA	GID	TG	DE	KK	SS	KG	PES	KEG	SG	NN	SR	GA	ENS	TM	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	TV	RG	NT	LN	DH	LL						
DeMV	SGNNNP	--	PE	VDA	GME	AG	KDE	KK	SS	KG	PES	KEG	SG	NN	SR	GA	ENS	PM	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	MP	VK	GN	MT	LN	DH	LL					
BCMV-US4	SGIGQ	PQ	PP	I	VDA	G	VE	SG	KDK	KE	SS	NK	KG	P	ED	RE	GA	SS	N	RE	AE	NS	SV	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	MP	VK	GN	MT	LN	DH	LL
BCMV-NL2	SGSNQ	PQ	PP	I	VDA	G	MD	AG	KDK	KE	SS	NK	KG	P	ES	RE	GA	SS	N	RE	AE	NS	SV	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	MP	VK	GN	MT	LN	DH	LL
PStv-OK	SGSST	TQ	SP	VL	DAG	VD	TAK	DK	KE	SS	NK	KG	P	ES	ES	SG	NN	SR	GA	ENS	TM	RDK	VD	NAG	SK	GR	VV	PR	LQ	RI	TR	KM	NP	MP	VK	GN	MT	LN	DH	LL		

Fig. 1. Alignment of the N-terminal CP amino acid sequences of BCMV-AzM-JH (this paper), BCMV-AzM-W (U60100), DeMV (U23564), BCMV-US4 (U37074), BCMV-NL2 (L19472), and PStV-OK (X63559). Dashes indicate gaps inserted to improve the alignment. Residues conserved in the majority of the viruses are in shaded boxes.

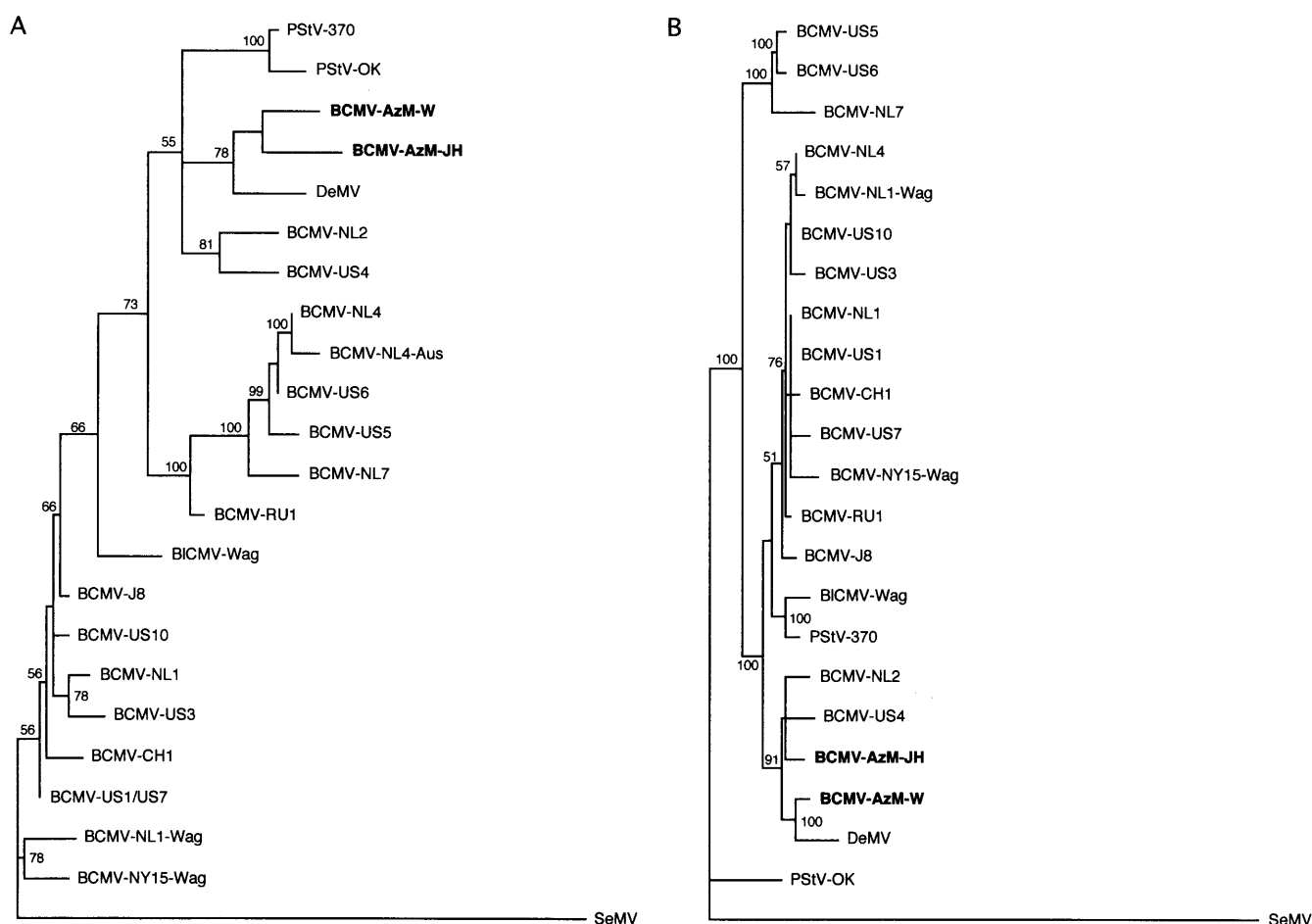


Fig. 2. Phylogenetic analysis of BCMV-AzM isolates and viruses in the BCMV group. A. Phylogenetic tree based on the amino acid sequences of the CP gene. B. Phylogenetic tree based on the nucleotide sequences of the 3' non-coding region. Branch lengths are proportional to distance, whereas the vertical position is arbitrary. Numbers on the trees represent the computed consensus using the 50% majority rule. The source of sequences is described by Berger *et al.*¹⁾. Sesame mosaic virus (U90326) is used as the outgroup.

virus sequence¹⁰⁾, is more highly conserved between BCMV-AzM-W and DeMV (88.0% identity) than between BCMV-AzM-JH and BCMV-AzM-W (78.6%). Furthermore, the two-amino acid deletion near the N-terminus was absent. The sequence RKMNP-V-GNM (amino acids 71-82) appears to be characteristic of AzM isolates. BCMV-US4, -NL2, and PStV-OK have CP amino acid sequence identities of 90.6% with BCMV-AzM-JH. BCMV-AzM-JH and bean common mosaic necrosis virus (BCMNV) strains -TN1 and -NL3 show 79.6 and 78.8% identities, respectively. Therefore,

BCMV-AzM-JH should be regarded as a strain of BCMV, not of BCMNV, in agreement with Shukla and Ward's proposal⁶⁾.

The 3' non-coding region contains 252 nucleotides, excluding the termination codon and poly(A) tail. It is one nucleotide shorter than those of BCMV-AzM-W, DeMV, PStV-OK, as are many BCMV strains. When this nucleotide sequence was compared with those of other viruses in the BCMV group, the identities ranged from 91.7% to 96.4%, and BCMV-NL1, -US1, and -NL2 showed the highest identities (96.4%). The sequence of

the 3' non-coding region is highly conserved among BCMV strains including the AzM strain.

A phylogenetic analysis was undertaken using previously described methods¹⁾. Multiple nucleotide or predicted amino acid sequences were aligned using the program PILEUP as implemented in the GCG package of software programs³⁾. Parsimony phylogenetic analyses of the aligned sequences were done using PAUP⁷⁾. The analyses using PILEUP and other software within the GCG suite of programs were performed at the VADMS Center (Washington State Univ.), and PAUP was run on a Macintosh computer. The branch lengths are proportional to the distance on all of the trees depicted, whereas the vertical position is arbitrary. The analysis generated a large number of equally parsimonious trees. A consensus was generated using the 50% majority rule. The branching patterns occurring more than 50% of the time are identified on the respective trees (Fig. 2). The phylogenetic tree based on the CP amino acid sequences (Fig. 2A) indicates that BCMV-AzM-JH and BCMV-AzM-W are isolates of the same strain and that they are closely related to DeMV. Analysis of the 3' non-coding region supports this conclusion, but BCMV-AzM-JH clusters with BCMV-US4 and -NL2 rather than with DeMV (Fig. 2B). Therefore, we propose that AzMV should be reclassified as the azuki bean mosaic strain of bean common mosaic virus (BCMV-AzM-JH and -W).

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和文摘要

高橋葉子・鎌形泰代・松村 健・上田一郎・Gaylord I. MINK・Philip H. BERGER: インゲンマメモザイクウイルスのアズキモザイク系統ゲノムの3'末端領域の塩基配列と、インゲンマメモザイクウイルスグループ内での系統学的関係

北大圃場より分離したインゲンマメモザイクウイルス (BCMV) アズキモザイク系統 (AzM-JH) のゲノムについて3'末端領域 2167 塩基の配列を決定した。外被タンパク質 (CP) のアミノ酸配列は、既報のワシントン分離株 (BCMV-AzM-W), dendrobium mosaic virus (DeMV) とそれぞれ 93.9%, 90.5% の高い相同性を示したが, BCMV-AzM-W と DeMV に共通する N 末端の 2 アミノ酸の欠失は見られなかった。3'末端非翻訳領域は保存性が高く, BCMV-AzM-JH と BCMV 各系統との間に 90% 以上の相同性があった。CP のアミノ酸配列と 3'末端非翻訳領域の塩基配列のそれぞれに基づいて, BCMV グループの系統解析をしたところ, BCMV-AzM-JH は BCMV-AzM-W, DeMV, BCMV-US4, BCMV-NL2 と一群をなし, これらは進化的に近縁であることが示された。

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