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Short Communication

Sequence Analysis of Expressed cDNA of *Bean Common Mosaic Virus* RU1 Isolate

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Bean common mosaic virus (BCMV) is a species of the genus *Potyvirus*. The genomes of potyviruses consist of a single, positive-sense RNA. A virus-encoded protein, VPg, is covalently linked to the 5' end of the genomic RNA and the 3' end is polyadenylated. The potyvirus genome is approximately 10,000 nucleotides with a single large open reading frame flanked by 5' and 3' untranslated regions. The open reading frame is translated as a large polyprotein, which is processed by three virus-encoded proteases. The resulting protein products are known as P1-proteinase (P1-pro), helper-component proteinase (HC-pro), P3 protein (P3), 6 kDa protein 1 (6K1), cytoplasmic inclusion protein (CI), 6 kDa protein 2 (6K2), virus protein genome linked (VPg), nuclear inclusion protein a proteinase (NiaPro), nuclear inclusion protein b RNA dependent RNA polymerase (Nib RdRp) and the coat protein (CP) (1). The species BCMV consists of several strains including *azuki bean mosaic virus, blackeye cowpea mosaic virus, dendrobium mosaic virus, peanut chlorotic ring mottle virus, peanut mild mottle virus,* and *peanut stripe virus* (2).

Five strains from *Phaseolus vulgaris* have been fully sequenced: strain NL1 (AY112735), strain NL4 isolate CIAT (DQ666332), strain blackeye cowpea (AJ312438) strain peanut stripe (AY968604) and the Russian strain RU1 (AY863025). In the present study we report the sequence of strain RU1 being infectious in a cDNA clone.

BCMV strain RU1 was kindly provided by Dr R. C. Larsen (USDA-ARS Prosser, WA, and USA) in seeds of *P. vulgaris* cultivar 'Black Turtle Soup II. Plants were grown from infected seed and RNA was isolated from leaves displaying mosaic symptoms. Overlapping fragments covering nucleotides 26 to 10002 of the virus genome were amplified by RT-PCR using M-MLV reverse transcriptase (Invitrogen, Carlsbad, US-CA) and *Pfu* polymerase (Stratagene, Amsterdam, The Netherlands) following the manufacturer's recommendations. PCR fragments originating from two independent RT reactions were cloned in TOPO ZeroBlunt vector (Invitrogen, Carlsbad, US-CA). Cloned cDNA fragments were analysed by restriction enzyme digestions and sequenced at Eurofins MWG operon (Ebersberg, Germany).

The sequence of BCMV RU1 with the NCBI nucleotide database accession number AY863025 was originally determined by Larsen *et al* (2005) for a study of recombination of the isolates RU1 and NL-3 D. Restriction digestions of the cDNA clones displayed some inconsistencies with the published sequence. Therefore, we sequenced the cloned cDNA of RU1. All nucleotides were determined twice on at least two clones

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Table 1. Non-silent nucleotide polymorphisms and affected amino acids differentiated AY863025 and sequence found for BCMV-RUI in this study (GQ219793).

Nucleotide number	Nucleotide		Amino acid		Nucleotide number	Nucleotide		Amino acid	
	AY863025	GQ219793	AY863025	GQ219793		AY863025	GQ219793	AY863025	GQ219793
ORF(141-9749)			P1					CI	
255	C	T	P	S	4134	T	A	W	R
652	A	G	D	G	4347	G	A	E	K
953	T	A	H	Q	5376	G	A	A	T
			HcPro					VPg	
2026	C	T	S	F	6088	A	G	K	R
2041	A	G	K	R	6141	C	G	R	G
2043	A	G	R	G	6153	C	A	P	T
2125-6	AA	GG	K	R	6169	C	A	P	Q
2166	G	A	V	I	6180	C	A	P	T
2179-80	AT	GC	N	S	6215	A	T	K	N
2217	A	G	I	V	6219	G	A	V	I
2232	G	T	V	L	6227	T	G	I	M
2421	A	G	T	A					
2615	T	G	D	E				NIaPro	
2676	A	T	T	S	7226	C	G	H	Q
2742	A	G	N	D	7239	A	G	T	A
					7264	A	G	N	S
			P3		7323	C	A	P	T
2928	T	A	L	M	7326	T	G	L	V
3033	G	A	D	N					
3288	A	G	K	E				NIb	
3705-7	AGG	GCA	S	A	7339-40	TC	GT	I	S
3708-10	ATT	TTA	I	L	7654	T	C	F	S
3711-13	AGG	GGA	R	G	7663	T	C	F	S
3714	A	G	R	G	7723	T	C	V	A
3717-19	GGC	CCT	A	P	8338	T	G	V	G
3747-9	ATT	CTC	I	L					
3777	G	A	V	I				CP	
3795	C	G	P	A	9022	G	A	S	N
					9093-5	CCC	ACA	P	T
			6K1		9099-9101	DEL	AGA	DEL	R
3870	C	G	P	A	9235-6	CT	GC	A	G
3948-50	ATA	CAT	I	H	9257	T	A	D	E
3951-3	CAT	AGA	H	T	9625	G	A	S	N
					9735-7	CCT	TCC	P	S

obtained from independent RT-reactions. This revealed 226 nucleotide differences compared with AY863025. Our revised sequence has been submitted to GenBank (GQ219793) and throughout this paper all the positions are numbered according to the GQ219793 sequence. Eighty-four of the nucleotide differences resulted in 57 coding differences (Table 1). Three nucleotides at positions 9099-9101 were absent in AY863025.

To confirm the authenticity of the revised sequence, the cloned cDNA was assembled in vector pAGUS1 as described (3) between the 35S promoter and NOS terminator. We had noticed that clones containing the region encoding the P3-6K2-CI region were unstable in *Escherichia coli*. Therefore intron IV2 of the *ST-LS1* gene from *Solanum tuberosum* (4) was inserted between nucleotides 4211-4212 using SOEING PCR (5, 6). The clone covering the 3' end of the genome was amplified with a primer

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containing 35 T's to assure a sufficiently long poly(A) tail. DNA of the full-length clone was manually inoculated to leaves of *P. vulgaris* cultivars Dubbele Witte and Stringless Green Refugee (7). Symptom appeared on inoculated leaves four days post inoculation and on uninoculated leaves 10 days later. Virus infection was confirmed by ELISA using antiserum raised against BCMV coat protein.

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