A New Strain of Bean Common Mosaic Virus From Lima Bean (*Phaseolus lunatus*): Biological and Molecular Characterization

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Abstract

Lima bean (*Phaseolus lunatus*) is a popular cultivated legume vegetable grown in the United States for dry bean or canned bean production. In 2017, two symptomatic *P. lunatus* plants exhibiting mosaic, vein banding, and growth retardation were collected in a public garden in Honolulu, HI. Both samples contained bean common mosaic virus (BCMV), and the two BCMV isolates were subjected to biological characterization on a panel of 11 differential cultivars of common bean (*P. vulgaris*), and to molecular characterization through whole genome sequencing. Both samples contained nearly identical BCMV sequences, named BCMV-A1, which, in turn, were 93% identical to the peanut stripe virus strain of BCMV. BCMV-A1 induced an unusually severe systemic necrosis in cultivar ‘Dubbele Witte’, and pronounced necrotic or chlorotic

reaction in inoculated leaves of five other bean differentials. BCMV-A1 was able to partially overcome resistance alleles *bc-1* and *bc-2* expressed singly in common bean, inducing no systemic symptoms. Phylogenetic analysis of the BCMV-A1 sequence, and distinct biological reactions in common bean differentials suggested that BCMV-A1 represented a new lima bean strain of BCMV. In 2017, two BCMV isolates were collected in Idaho from common bean, and based on partial genome sequences were found 99% identical to the BCMV-A1 sequence. The data suggest that the lima bean strain of BCMV may have a wider circulation, including common bean as a host. This new strain of BCMV may thus pose a significant threat to common bean production.

Bean common mosaic virus (BCMV) is an important pathogen affecting common bean and other legumes. BCMV is an aphid-transmitted virus, which can also be seed-transmissible in common bean with efficiencies of up to 80%, depending on virus strain and bean cultivar (Drijfhout and Morales 2005). The virus belongs to the genus *Potyvirus* (family *Potyviridae*), having an approximately 10-kb single-stranded, positive-sense RNA genome (Morales and Bos 1988; Wylie et al. 2017). BCMV resistance in common bean is governed by one dominant (*I*) and four recessive (*bc-u*, *bc-1*, *bc-2*, and *bc-3*) genes which are deployed by breeders in different combinations to protect commercial bean cultivars against the virus (Drijfhout 1978; Kelly et al. 1995; Meziadi et al. 2017; Singh and Schwartz 2010). Other crop legumes can be susceptible to BCMV, such as peanut (*Arachis hypogaea* L.; Demski et al. 1984; Gunasinghe et al. 1994), cowpea (*Vigna unguiculata* subsp. *unguiculata*; Purcifil and Gonsalves 1985; Wang and Fang 2004), soybean (*Glycine max* L.; Zhou et al. 2014), and azuki bean (*Vigna angularis* Ohwi & Ohashi; Li et al. 2016), but genetics of BCMV resistance in these legumes has not yet been elucidated. Wild legume hosts, including *V. trilobata* and *Macroptilium atropurpureum*, were also found supporting systemic infection of BCMV representing a potential reservoir of the virus (Courtts et al. 2011).

BCMV exists as a complex of strains exhibiting significant biological and genetic diversity. At least eight biological pathotypes or strains of BCMV have been defined based on their interactions with 11–12 differential lines of common bean harboring different combinations of resistance alleles (Drijfhout 1978; Drijfhout and Morales 2005; Feng et al. 2015). BCMV strains were also found to display a significant genetic diversity, correlating with host specificity (Berger et al. 1997; Gibbs et al. 2008; Zhou et al. 2014), and often having recombinant genomes (Feng et al. 2014a, b, 2015). Several host-related lineages of BCMV were defined, with at least two originating in common bean (US1/NL1 and RU1; Feng et al. 2014b, 2015, 2018; Larsen et al. 2005; Naderpour et al. 2009), one in peanut (PStV; Gunasinghe et al. 1994; Zhou et al. 2014), one in blackeye cowpea (BICMV; Wang and Fang 2004; Zhou et al. 2014), one in soybean (Zhou et al. 2014), and one in azuki bean (Berger et al. 1997; Li et al. 2016). The nucleotide sequence identity between these BCMV lineages varies between 82 and 94% for the whole genome (Feng et al. 2014a, b, 2015, 2018; Larsen et al. 2005; Li et al. 2016; Wang and Fang 2004; Zhou et al. 2014). One BCMV partial sequence, FL-1, from *Macroptilium atropurpureum* (Courtts et al. 2011), was found distant from other known BCMV isolates displaying 92% or less nucleotide sequence identity.

Lima bean (*Phaseolus lunatus* L.) is a popular cultivated legume vegetable grown in the United States for dry bean or canned bean production. In the United States, it is a relatively new legume crop, and viruses infecting *P. lunatus* are not well studied; importantly, the strains of BCMV circulating in lima bean have not yet been characterized. Recently, lima bean plants grown in a community garden in Honolulu, HI, were reported to exhibit stunting symptoms as well as mosaic, mottling, and chlorosis of the leaves and were found to be infected with BCMV (Green et al. 2017); however, the strain of BCMV was not identified. In South America and in Africa, *P. lunatus* was reported to be infected with BCMV (Melgarejo et al. 2007; Sengooba et al. 1997), and strain composition of BCMV isolates was found similar to common bean strains of the virus (Melgarejo et al. 2007).

Here, we described a new strain of BCMV from lima bean with distinct biological and molecular characteristics found in a community garden in Honolulu, HI. This new BCMV strain, BCMV-A1,

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Funding: This work was funded, in part, through grants from the Idaho Bean Commission, the Idaho State Department of Agriculture, the USDA National Institute of Food and Agriculture (Hatch project IDA0560); and by the Idaho Agricultural Experiment Station.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

The authors declare no conflict of interest.

Accepted for publication 10 January 2019.

was able to partially overcome resistance to the virus conferred by *bc-1* and *bc-2* alleles in common bean, establishing an asymptomatic systemic infection in some common bean cultivars. Later, two BCMV isolates were found in commercial common bean samples in Idaho exhibiting 99% identity to BCMV-A1 in partial genome sequence. This new BCMV strain from lima bean, BCMV-A1, may present a threat for common bean production.

Materials and Methods

Virus sources and maintenance. In May 2017, BCMV isolates A1 and A2 were collected from two symptomatic *P. lunatus* plants exhibiting leaf mosaic, vein banding, and growth retardation, at the same community garden in Honolulu, HI, where BCMV was reported earlier (Green et al. 2017). Potyvirus infection in these two plants was determined using a lateral flow assay (Agdia, Elkhart, IN), and leaf samples were transferred to the University of Idaho under USDA permit. BCMV isolates F17:0298A and F17:0298D were collected in Boise, ID, from two *P. vulgaris* plants exhibiting seed-borne infection, and provided by Elizabeth Vavricka (Idaho State Department of Agriculture) in August 2017. The origin of reference BCMV isolates RU1-P, RU1-OR, and 1755a was described previously (Feng et al. 2014b, 2015). All virus isolates were propagated in the bean cultivar ‘Dubbele Witte’ or in *Nicotiana benthamiana* using mechanical inoculation and maintained under greenhouse conditions as described previously (Feng et al. 2014b).

Biological and serological characterization. The biological characterization of BCMV isolate A1 and A2 on a set of 11 bean differentials (Drijfhout 1978) was performed as described previously for BCMV typing (Feng et al. 2014b). Three reference strains, RU1-P, RU1-OR, and 1755a, were included in this analysis as controls. All 11 bean lines were inoculated with each isolate (two or three plants per cultivar), and plants were kept in a climate-controlled growth room with standard summer-time growth conditions (16-h day photoperiod and daytime/nighttime temperatures of 25/16°C). Symptoms in inoculated and systemic noninoculated leaves were recorded at weekly intervals, up to 5 weeks postinoculation (wpi). For *I* gene containing cultivars including ‘Jubila’, ‘Amanda’, ‘US1006’, and ‘IVT7233’, 2 to 3 leaf samples were randomly collected from the upper noninoculated leaves at 5 wpi and tested by triple-antibody sandwich (TAS) ELISA. For the rest of the differential lines lacking *I* gene, and carrying combined recessive alleles, samples from each single-inoculated and upper-noninoculated leaf were collected and tested at 3 wpi and 5 wpi, respectively, to assess the nature of recessive resistance gene conferred against the lima bean isolate under study. The

presence or absence of the virus in leaf tissue was confirmed by TAS-ELISA using BCMV-specific, polyclonal antibodies from the laboratory collection as described previously (Feng et al. 2014b).

Sequencing and sequence analysis. The whole genomes of BCMV isolates A1 and A2 were sequenced directly from overlapping RT-PCR fragments amplified using a set of primers developed for this study (Table 1). The partial genome sequences of BCMV isolates F17:0298A and F17:0298D were also amplified using BCMV-specific primers by RT-PCR as described (Table 1; Feng et al. 2014b, 2015). The PCR profile consisted of denaturing at 94°C for 2 min; 35 cycles of 94°C for 20 s, 50–55°C for 30 s (depending on the melting temperatures of primers used); and 72°C for 1–1.5 min (depending on the fragment length amplified); followed by a final extension for 10 min at 72°C. The PCR amplicons were treated with Exosap-It (Affymetrix, Cleveland, OH) and submitted for sequencing to Elim Biopharmaceuticals (Hayward, CA) as described (Feng et al. 2014b). The 5′-terminal sequence for A1 and A2 isolates was amplified using the RACE Kit (Roche), and subsequently cloned and sequenced the same way as described previously (Feng et al. 2014b). The complete and partial viral genome sequences were assembled using SeqMan (DNASTAR, Madison, WI) and deposited in the GenBank database under accession numbers MK282414 and MK282413 (BCMV-A1 and BCMV-F17:0298A, respectively). The sequences were initially analyzed using the BLASTn 2.2.17 tool (Altschul et al. 1997) available at the National Center for Biotechnology Information (NCBI). Open reading frames (ORFs) were identified using the ORF Finder program available at the NCBI. Complete genome of BCMV-A1 was aligned with 11 other BCMV isolates and one BCMNV isolate using ClustalX Ver. 2.0 (Conway Institute, UCD, Dublin). Among 12 BCMV isolates were two BCMV-PStV isolates, two soybean isolates, two RU1 type, and another two US1 type BCMV isolates. Additionally, two were BCMV-BICMV isolates, one an adzuki bean isolate, and BCMV-A1 representing a lima bean isolate. The partial genome sequence of BCMV-F17:0298A was aligned with the same 12 BCMV isolates mentioned above, and an isolate of the closely related potyvirus, bean common mosaic necrosis virus (BCMNV), using ClustalX Ver. 2.0, and all were trimmed to equal size (699 nt long). Based on the obtained alignment of 12/13 BCMV complete or partial genomic sequences, Maximum-Likelihood (ML) phylogenetic trees were generated in MEGA 6 (Tamura et al. 2013). To test the robustness of an individual branch, 100 replicates were used to perform bootstrap analysis. BCMNV (HQ229995) was used as an outgroup species. PStV-JX014 (KJ807813), BICMV (AY575773), RU1-P (KF919300), and US1 (KT175569) sequences were aligned

Table 1. Primers used for sequencing of bean common mosaic virus (BCMV) isolate A1 and BCMV-ID genomes

Primer	Position	Sequence
<i>Whole genome sequencing</i>		
SP F1	1	AATTAACCAACTCATAAAGAC
SP R1	1514	GCCCACTGTGCATTGTC
SP F2	1351	GGTGAATGCACCTGGATGAAC
SP R2	2880	CCCATCAGTAGGATATAGGG
SP F3	2774	GATGCACAACAAAGAATGCG
SP R3	4053	GTGAAGTAGCTTCCTTGC
SP F4	3685	GTGTTGCAAAGTCACAGGT
SP R4	5333	CCATTCTGATTCTCTCAGC
SP F5	5157	CAACACAAGGTGTCACATC
SP R5	6283	CAAAGTTGCACCAAGTGAGTG
SP F6	6089	CGCGAGGTGTATGCTGATG
SP R6	7681	GTATATCTCCTCTGGATCTG
SP F7	7399	GGTGACAAAGCATGTAGTG
SP R7	8864	CAGATTCTCCACATCCAAC
SP F8	8628	GTGCAGCAATGATTGAAGC
Ancor-8R	3′ end of genome	GACCACGCGTATCGATGTGCGAC
first strand RT	3′ end of genome	GACCACGCGTATCGATGTGCGAC(T)17A
SP 5RACE-PCR	214	CTATGAGCTCTTCGACTTCC
<i>Partial genome sequencing</i>		
BCMV-NL1-1950F	1925	CGGAGGCTGAYCCACTGAAGAC
BCMV-NL1-2700R	2623	CCTGTGGTGAGGGATCCAAAT

with BCMV-A1, and further analysis was conducted with Recombination Detection Program v.4.16 (RDP4) (Martin et al. 2005).

Results

Collection of samples and maintenance of the lima bean isolates.

The two BCMV-infected samples, named A1 and A2, were collected from lima bean plants grown in a community garden in Honolulu, HI. Both infected plants exhibited stunting, mosaic, mottling, and chlorosis of the leaves. The community garden where the BCMV-infected bean plants were observed was directly adjacent to a canal and consisted of 153 fenced plots (~4 × 5 m). A large number of tropical and temperate horticultural and crop species as well as weed species were grown here, and the access for the public was unrestricted. Once determined to be infected with a potyvirus, the infected material was shipped to the University of Idaho Plant Virology Laboratory for further analysis, and the BCMV infection of both samples was confirmed using the BCMV-specific TAS-ELISA assay (Feng et al. 2014a).

The A1 and A2 isolates were initially transferred to and propagated in ‘Dubbele Witte’ to provide infectious material for the biological tests in bean differentials. However, maintenance of both A1 and A2 isolates of BCMV in ‘Dubbele Witte’ proved difficult, as both induced systemic necrosis and death of the infected plant in about 3 wpi (see below). Both isolates were tested for infectivity in *N. benthamiana* and found to induce mild mosaic, blistering, leaf deformation, and growth retardation visible at 2–3 wpi, with no

necrotic reaction visible even at 8 wpi (not shown). *N. benthamiana* was used as an alternative source of BCMV-A1/A2 inoculum in some experiments.

Pathogenicity testing in common bean cultivars harboring or lacking the *I* gene. When subjected to a standard pathotyping on 11 differential lines of common bean, both A1 and A2 isolates were tentatively assigned to pathogroup I (PG-I), since only two cultivars, ‘Dubbele Witte’ and ‘Stringless Green Refugee’ exhibited systemic symptoms in upper, noninoculated leaves (Supplementary Table S1). Nevertheless, ELISA testing of the randomly selected upper, noninoculated leaves of three inoculated cultivars, ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’ suggested asymptomatic infection in some of the tested leaves in these cultivars inoculated with BCMV-A1 and A2. A more thorough experiment was therefore conducted with BCMV-A1 inoculated into seven common bean cultivars from the differential set, those not carrying the *I* gene (Table 2). In inoculated leaves of most cultivars lacking *I* gene, A1 and A2 isolates of BCMV induced chlorotic spots at around 2 wpi (11–14 dpi) (Fig. 1). Later, leaf yellowing and vein necrosis started to develop at around 3 wpi (18–21 dpi) (Fig. 1). In ‘Dubbele Witte’, the plants were severely stunted as the result of the virus infection (Fig. 2); this stunting became very prominent as early as 13 dpi (Fig. 2). By 18 dpi, the newly developed upper noninoculated leaves exhibited severe systemic vein necrosis, and the infected plant wilted and died at around 3 wpi (Fig. 2).

Table 2. Resistance of bean differentials inoculated with bean common mosaic virus (BCMV) isolates^a

Bean cultivar	Resistance genes	A1 (PG-I)	RU1-P (PG-VI)	RU1-OR (PG-VII)	1755a (PG-VIII)
‘Dubbele Witte’	None	+/S	+/S	+/S	+/S
‘SGR’	<i>i, bc-u</i>	+/S	+/S	+/S	+/S
‘RGLC’	<i>i, bc-u, bc-1</i>	-/PR	+/S	+/S	-/R
‘RGLB’	<i>i, bc-u, bc-1²</i>	-/PR	+/S	+/S	-/PR
‘Sanilac’	<i>i, bc-u, bc-2</i>	-/PR	+/S	-/PR	+/S
‘UI35’	<i>i, bc-u, bc-1², bc-2²</i>	-/R	-/PR	+/PR	-/R
‘IVT7214’	<i>i, bc-u, bc-2, bc-3</i>	-/IM	-/IM	-/IM	+/S

^a Disease reaction is measured by symptom observation and TAS-ELISA assessment of BCMV isolates for infection of both local inoculated and upper noninoculated leaves in different genotypes. Two or three plants were inoculated for each BCMV isolate per experiment, local inoculated leaves were tested at 3 wpi and upper noninoculated leaves were tested at 5 wpi; numerator: + = symptoms on inoculated beans; - = no symptoms on inoculated beans; denominator: S designates viruses were able to replicate and spread to the entire plant; IM designates no viruses were found from both local inoculated leaves and upper noninoculated leaves; R designates viruses were found replicating and moving normally in local inoculated leaves but unable to move beyond the inoculated leaf; PR designates viruses were able to replicate and move from cell to cell but their long-distance movement ability was impaired and could only be found in several upper noninoculated leaves.

13 dpi

19 dpi

DW

SGR

RGLC

RGLB

Sanilac

UI35

IVT7214

Fig. 1. Symptoms exhibited by the bean common mosaic virus isolate A1 on inoculated leaves of seven common bean (*Phaseolus vulgaris*) cultivars. These are ‘Dubbele Witte’ (DW), ‘Stringless Green Refugee’ (SGR), ‘Redlands Greenleaf C’ (RGLC), ‘Redlands Greenleaf B’ (RGLB), ‘Sanilac’, ‘UI35’, and ‘IVT7214’. Symptoms were observed at 13 days postinoculation (dpi, upper row), and at 19 dpi (lower row).

In these experiments, two primary, inoculated leaves per plant were tested by ELISA at 3 wpi to determine if BCMV-A1 can replicate and move cell-to-cell (Sulzinski and Zaitlin 1982), and in addition, all upper, noninoculated leaves from each of the inoculated plants were tested by ELISA at 5 wpi (at 3 wpi for ‘Dubbele Witte’ due to the severe necrosis of the plant) to determine if BCMV-A1 can move systemically in the inoculated plants (Table 3). Interestingly, BCMV-A1 was found able to replicate and move cell-to-cell in inoculated leaves of six out of the seven tested cultivars, apparently unable to overcome only the *bc-3* gene in ‘IVT7214’ (Table 3). This ability to replicate and move in inoculated leaves in the presence of all alleles of *bc-1* and *bc-2* genes was similar to the properties of other BCMV strains from common bean (Feng et al. 2018). Not surprisingly, BCMV-A1 was found invading all noninoculated leaves of the two cultivars where it exhibited systemic symptoms, ‘Dubbele Witte’ and ‘Stringless Green Refugee’ (Table 2), both cultivars carrying either no recessive resistance genes or just a helper gene *bc-u*, respectively. But BCMV-A1 was also able to invade systemically various proportions of noninoculated leaves in three cultivars carrying single *bc-1* or *bc-2* alleles, ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’ (Table 2). Cultivar ‘UI-35’, carrying a combination of *bc-1* and *bc-2* alleles, supported the replication and cell-to-cell movement of BCMV-A1 in inoculated leaves but did not permit its systemic spread (Table 2).

Whole genome sequencing and sequence analysis. The assembled overlapping amplicons of BCMV isolates A1 and A2 were both found to be 9,995 nucleotides (nt) long, excluding the poly(A). The A1 and A2 sequences were nearly identical ($\approx 99.7\%$ nt identity), and a single name was retained, BCMV A1. A single A1 sequence was deposited into GenBank under accession number MK282414. Based on conceptual translation, the BCMV A1 genome encoded a single polypeptide of 3,202 aa. The sequence of A1 was compared with the known BCMV genomes using the BLAST tool, and found to share the closest identity (93%) with BCMV isolate PStV-JX014 (accession number KJ807813).

To infer phylogenies between BCMV-A1 and other BCMV strains, 12 complete genomes of BCMV representing lineages associated with different host species were aligned; these host species included peanut, soybean, common bean, blackeye cowpea, azuki bean, and lima bean. The overall phylogenetic grouping of BCMV strains (Fig. 3) was consistent with the phylogenies generated on a smaller number of BCMV strains (Zhou et al. 2014). Indeed, all three clades comprising soybean (clade I), peanut (II), and common bean (III) strains of BCMV (Zhou et al. 2014) could be easily identified (Fig. 3). BCMV-A1 was placed closer to the peanut lineage of BCMV, but as a distinct sequence, while the relatively new azuki bean strain appeared closer to the common bean lineage comprising US1 and NL1 strains (Fig. 3). In pair-wise comparisons, BCMV isolates from clade I shared 86% nucleotide sequence identity with clade II and 84 to 86% sequence identity with clade III. Clades II and III shared 83 to 85% nucleotide sequence identity.

To visualize the distribution of the genetic diversity along the entire genome, the whole genomes for BCMV-A1 and four other known BCMV strains were aligned and subjected to recombination analysis. Figure 4A shows the comparison of the five BCMV sequences using the manual distance plot analysis, with the full-length A1 sequence used as reference. The whole genomes of BCMV-Az (accession number KP903372) and DXH025 (accession number KP807812) were not included in this analysis, as lines representing their genomes closely overlapped with the line representing US1 genome. As can be seen from Figure 4A, most of the sequence diversity between BCMV isolates studied was in the 5'-UTR and in the adjacent P1 area, nucleotides 1 to 935.

Screening of the common bean BCMV isolates from Idaho and partial sequence analysis. In August 2017, five samples were submitted to the UI Virology Laboratory from the Idaho State Department of Agriculture for BCMV and BCMNV testing. The five foliar samples were from five individual bean seed lots suspected of BCMNV infection. Following the TAS-ELISA testing (Feng et al. 2014b), the BCMNV infection of the samples was excluded, and

all five samples were confirmed to be infected with BCMV (not shown). Due to the poor condition of samples, the attempts to mechanically inoculate BCMV isolates F17:0298A and F17:0298D into ‘Dubbele Witte’ were not successful.

The partial, 699-nt long genome fragments of BCMV isolates F17:0298A and F17:0298D were amplified, and only three nucleotide differences were found between the two fragments spanning the HC-Pro cistron (Fig. 4A). The nucleotide sequence for the F17:0298A isolate was renamed BCMV-ID and compared with the known BCMV and BCMNV sequences. To our surprise, BCMV-ID was found sharing the 99% identity with isolate BCMV-A1. To examine the phylogenetic position of the BCMV-ID sequence further, this same genome fragment of HC-Pro from 12 BCMV isolates analyzed in Figure 3 was aligned using ClustalX, and phylogenies were inferred using BCMNV-TN1 sequence (accession number HQ229995) as out-group. The relationships between strains of BCMV based on the partial genome analysis of the HC-Pro region (Fig. 4B) were consistent with phylogenies based on whole genome sequence of the same 12 BCMV strains (Fig. 3). At the same time, the BCMV-ID sequence was placed in a tight lineage with the lima bean strain BCMV-A1, in the same clade with the peanut strain of the virus (Fig. 4B).

Discussion

The genetic diversity of BCMV was found to generally correlate with the host specificities of the isolates studied (Gibbs et al. 2008; Li et al. 2016; Zhou et al. 2014), resulting in distinct phylogenetic clades corresponding to several legume species supporting systemic infection of the virus. For common bean isolates of BCMV, a differential panel of reference cultivars of *P. vulgaris* was established allowing typing of common bean isolates of the virus according to their interactions with five BCMV resistance genes (Drijfhout 1978; Drijfhout and Morales 2005; Feng et al. 2015). Eight

Fig. 2. Systemic symptoms induced by the bean common mosaic virus (BCMV) isolate A1 in common bean (*Phaseolus vulgaris*) cultivar ‘Dubbele Witte’ (DW) at 13 and 18 days postinoculation (dpi); arrows point at inoculated leaves.

pathogroups or strains of BCMV were established for common bean isolates (Drijfhout 1978; Feng et al. 2015), which could be phylogenetically separated into two clades based on sequence comparisons, and typified by US1 and RU1 isolates (Feng et al. 2015; Larsen et al. 2005). In addition to these two common bean BCMV clades, multiple recombinants of these two parental sequences were described, as well as recombinants with genome segments from still unknown

parental sequences (Feng et al. 2014a, 2014b, 2015). However, the biological diversity of the BCMV isolates originating from legume species other than common bean (*P. vulgaris*) was poorly studied up until now.

BCMV-A1 is the first BCMV isolate from lima bean subjected to a complete biological and molecular characterization. The previously reported BCMV isolate collected in 2016 from lima bean in Hawaii

Table 3. Biological characterization of the bean common mosaic virus (BCMV) isolate A1 on common bean differentials

Cultivar/resistance gene(s) ^a	Plant	Inoculated leaves, 2 wpi ^b		Upper, noninoculated leaves, 5 wpi ^b		Symptoms ^c	
		Positive/tested	Percent positive	Positive/tested	Percent positive	IL	NIL
DW/none	1	2/2	100%	NA	NA	NLL	syst. N
	2	2/2	100%	NA	NA		
	3	2/2	100%	NA	NA		
SGR/ <i>bc-u</i>	1	2/2	100%	33/34	97%	NLL	MM, VN
	2	2/2	100%	27/27	100%		
	3	2/2	100%	28/28	100%		
RGLC/ <i>bc-u</i> , <i>bc-1</i>	1	2/2	100%	2/24	8%	ClSp	NS
	2	2/2	100%	11/30	37%		
	3	2/2	100%	8/27	30%		
RGLB/ <i>bc-u</i> , <i>bc-1</i> ²	1	2/2	100%	18/32	56%	ClSp	NS
	2	2/2	100%	12/24	50%		
	3	2/2	100%	12/25	48%		
Sanilac/ <i>bc-u</i> , <i>bc-2</i>	1	2/2	100%	35/37	95%	ClSp	NS
	2	2/2	100%	30/32	94%		
	3	2/2	100%	6/21	29%		
UI-35/ <i>bc-u</i> , <i>bc-1</i> ² , <i>bc-2</i> ²	1	2/2	100%	0/37	0%	NLL, VN	NS
	2	2/2	100%	0/43	0%		
	3	2/2	100%	0/26	0%		
IVT 7214/ <i>bc-u</i> , <i>bc-2</i> , <i>bc-3</i>	1	0/2	0%	0/31	0%	NS	NS
	2	0/2	0%	0/38	0%		
	3	0/2	0%	0/33	0%		

^a DW, 'Dubbele Witte'; SGR, 'Stringless Green Refugee'; RGLC, 'Redland Greenleaf C'; RGLB, 'Redland Greenleaf B'.

^b wpi, weeks postinoculation.

^c IL, inoculated leaf; NIL, upper noninoculated leaf; NLL, necrotic local lesions; syst. N, systemic necrosis; MM, mild mosaic; VN, vein necrosis; ClSp, chlorotic spots; NS, no symptoms.

Fig. 3. Maximum-likelihood phylogenetic tree for the whole genomes of select bean common mosaic virus (BCMV) isolates. Sequence alignment was done using ClustalW provided in MEGA 4, and the tree was drawn using the RDP 4 program. Bootstrap values higher than 70% are shown at the corresponding nodes. Abbreviations of the BCMV isolate names are followed by the corresponding GenBank numbers. Light brackets indicate lineages of US1, RU1, BICMV, Soybean, and PSTV, associated with strain groups, while black brackets indicate main evolutionary lineages as indicated by Zhou et al. (2014). Bean common mosaic necrosis virus (BCMNV), isolate TN1 sequence was used as an outgroup.

(Green et al. 2017) was only partially sequenced, and only approximately 350-bp spanning the NIb cistron is available for comparisons (accession number KY473075). Nevertheless, this partial sequence exhibited only 87% identity with the BCMV-A1 sequence at the

nucleotide level (not shown), suggesting substantial diversity of BCMV isolates circulating in lima bean in Hawaii. Five isolates of BCMV from lima bean were collected in Peru previously, and characterized through partial sequencing (Melgarejo et al. 2007). One of

Fig. 4. Distribution of the genetic diversity along the whole genome of the bean common mosaic virus (BCMV) isolate A1 (**A**), and phylogenetic analysis of the partial genome sequence of the BCMV-ID isolate (**B**). (**A**) Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates from the main BCMV lineages; BCMV-A1 sequence was used as a reference. The horizontal double-arrow indicates the position of the BCMV-ID genome fragment analyzed in panel B. (**B**) Maximum-likelihood phylogenetic tree for the genome segment (nt 1973-2683) of select BCMV isolates, plus a partial sequence of the corresponding genome fragment for BCMV-ID. Position of the analyzed genome segment is marked with double arrow on panel A, above the distance plot. Sequence alignment was done using ClustalW provided in MEGA 4, and the tree was drawn using the RDP 4 program. Bootstrap values higher than 70% are shown at the corresponding nodes. Abbreviations of the BCMV isolate names are followed by the corresponding GenBank numbers. Light brackets and arrows indicate lineages of US1, RU1, BICMV, Soybean, and PStV, associated with strain groups shown in Figure 3. Bean common mosaic necrosis virus (BCMN), isolate TN1 sequence was used as an outgroup.

these lima bean isolates, LM1, was subjected to a host range study which included an incomplete panel of differential bean cultivars, and found to exhibit a unique set of properties indicating a possible new strain of the virus (Melgarejo et al. 2007). Because the bean differential panel was incomplete, LM1 isolate of BCMV could not be typed to strain, but partial nucleotide sequence of LM1 genome was found 98% identical to the US1 sequence from a common bean BCMV (Melgarejo et al. 2007). LM1 exhibited a much lower nucleotide sequence identity of 88% to the BCMV-A1 sequence described here. Partial CP cistron sequences for other lima bean isolates from Peru formed a distinct clade in the phylogenetic tree, but were not biologically typed (Melgarejo et al. 2007); these partial sequences were only 85% identical to our BCMV-A1 sequence. Although lima bean isolates of BCMV, LM1 and A1, were collected from different geographic locations, both were able to infect multiple bean cultivars, which indicated their ability to cause damage in *P. vulgaris*. Based on the low nucleotide sequence identities of BCMV-A1 to the other BCMV isolates from lima bean reported previously (Melgarejo et al. 2007), we concluded that BCMV-A1 isolate represented a possible new, unique strain of BCMV. The pathotype assignments and molecular characterization of BCMV isolates from lima bean will require further studies.

The rapid systemic necrotic reaction induced by BCMV-A1 in ‘Dubbele Witte’ (Fig. 2), and a less severe systemic necrosis in ‘Stringless Green Refugee’ (Table 2), both developing below 30°C, is an interesting phenomenon which is not characteristic of BCMV isolates from common bean, or even of *Bean common mosaic necrosis virus* (Drijfhout 1978; Drijfhout and Morales 2005). Similar necrotic reaction is more characteristic of *I*-gene harboring common bean cultivars without other, recessive resistance alleles when a plant is infected with BCMNV and undergoes various types of systemic necrosis (Ali 1950; Cadle-Davidson and Jahn 2005; Collmer et al. 2000; Drijfhout 1978; Feng et al. 2017). Neither ‘Dubbele Witte’, nor ‘Stringless Green Refugee’ has the *I* gene in their genetic background, however (Drijfhout 1978; Drijfhout and Morales 2005; Feng et al. 2017). It is tempting to speculate that both ‘Dubbele Witte’ and ‘Stringless Green Refugee’ may carry a new, putative resistance allele recognizing BCMV-A1 and inducing a HR response visible as systemic necrosis. This hypothesis will need to be tested through genetic crosses and analysis of segregating progeny populations. BCMV-A1 genetic determinants involved in overcoming the *bc-1* and *bc-2* resistance genes will need to be identified through reverse genetics experiments.

The three common bean cultivars, ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’, that permit systemic infection in 37 to 95% of noninoculated leaves can thus be considered partially resistant but not sensitive to BCMV-A1, according to the definition of reactions of plants to viruses (Cooper and Jones 1983). The fact that BCMV-A1 can establish an asymptomatic infection in some common bean cultivars (Table 2) suggested that this lima bean isolate could potentially threaten *P. vulgaris*. Indeed, our finding of a closely related BCMV-ID sequence in common bean samples from Idaho provided a direct evidence of this threat (Fig. 4B). It is possible that BCMV-A1 circulates in common bean production areas often remaining asymptomatic. Due to this threat, the lima bean strain of BCMV should be tested for by the seed certification agencies involved in BCMV diagnosis, and bean breeders need to consider this new BCMV strain when screening their material for resistance to BCMV.

Acknowledgments

We thank Dr. E. Vavricka for providing BCMV-positive samples, and J. Chojnacki for laboratory and greenhouse help.

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