Determining the mechanism of the recessive resistance to *Bean common mosaic virus* (BCMV) conferred by the *bc-1* and *bc-2* recessive genes in common bean, and biological and molecular characterization of a new strain of BCMV

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Authorization to Submit Thesis

This thesis of Gardenia Edith Orellana Arreaga, submitted for the degree of Master of Science with a Major in Plant Science and titled "**Determining the mechanism of the recessive resistance to *Bean common mosaic virus* (BCMV) conferred by the *bc-1* and *bc-2* recessive genes in common bean, and biological and molecular characterization of a new strain of BCMV**," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

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. The virus belongs to the genus *Potyvirus* (family *Potyviridae*), having an approximately 10-kb single-stranded, positive-sense RNA genome. BCMV resistance in common bean is governed by one dominant (*I*) gene 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. The dominant *I* gene confers extreme resistance or immunity against all strains of BCMV when the temperature stays below 30°C, and variable types of local and systemic necrosis when temperature exceeds 30°C. The *bc-3* gene was identified as an eIF4E translation initiation factor gene mediating resistance through disruption of the interaction between this protein and the VPg protein of the virus. The mode of action of *bc-1* and *bc-2* in expression of BCMV resistance was unknown until this work. To investigate the possible role of both *bc-1* and *bc-2* genes in replication, cell-to-cell, and long distance movement of BCMV in *P. vulgaris*, we tested virus spread of eight BCMV isolates representing pathogroups I, IV, VI, VII, and VIII, in a set of bean differentials expressing different combinations of six resistance alleles including *bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3*. The data suggest that *bc-1* and *bc-2* recessive resistance genes have no effect on the replication and cell-to-cell movement of BCMV, but affect systemic spread of BCMV in common bean. The BCMV resistance conferred by *bc-1* and *bc-2* and affecting systemic spread was found only partially effective when these two genes were expressed singly. The efficiency of the restriction of the systemic spread of the virus was greatly enhanced when the alleles of *bc-1* and *bc-2* genes were combined together.

Beside common bean, there are other crop legumes that are susceptible to BCMV, such as peanut, soybean, and azuki bean. In this thesis, I described a new strain of

BCMV from lima bean with distinct biological and molecular characteristics found in Honolulu, HI. This new BCMV strain, BCMV-A1, 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 the common bean production.

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Besides my advisor, I would like to thank Dr. Xue Feng. Her enthusiasm and passion for science made a strong impression on me and I will always carry positive memories of her teaching with me.

Several field isolates of *Bean common mosaic virus* (BCMV) used in this research were provided by Dr. Pablo Guzman (California Crop Improvement Association, Davis, CA), Prof. James Myers (Oregon State University, Corvallis, OR), Julie Thayer (Washington State University, Pullman, WA), and Dr. Michael Melzer (University of Hawaii at Manoa, Honolulu, HI). One isolate of BCMV, Viva2, was collected in LaConner, WA, by Dr. Xue Feng, then at University of Idaho (Moscow, ID), from a symptomatic common bean plant.

I would also like to show gratitude to my thesis committee: Prof. Brenda Schroeder, Prof. James Myers and Elizabeth Vavricka.

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Dedication

This thesis is wholeheartedly dedicated to my parents and to my brother, who have been my source of inspiration and continuous encouragement throughout my years of study.

Table of Contents

List of Figures

List of tables

CHAPTER 1: *BEAN COMMON MOSAIC VIRUS* (BCMV) - BRIEF SUMMARY

Bean common mosaic virus (BCMV) is a member of the genus *Potyvirus*, having a single-stranded, positive-sense RNA genome of approximately 10-kb, excluding poly(A) at the 3'-terminus, with a virus-encoded VPg protein covalently attached to the 5'-terminus (Adams et al. 2012). The virus genome encodes a single polyprotein that is co- and post-translationally cleaved by three virus-specific proteases into 10 mature proteins (Adams et al. 2012), and an out of-frame, small non-structural protein PIPO (Chung et al. 2008; Wei et al. 2010).

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; Feng et al. 2015). BCMV strains were also found to display a significant genetic diversity, correlating with host specificity, and often having recombinant genomes. Several host-related lineages of BCMV were defined, with at least two originating in common bean (US1/NL1 and RU1; Larsen et al. 2005; Naderpour et al. 2009; Feng et al. 2014b, 2015, 2018), one in peanut (PStV; Gunasinghe et al. 1994; Zhou et al. 2014) one in blackeye cowpea (BlCMV; 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. 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 U.S., 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).

Resistance to BCMV in common bean is governed by one dominant *I* gene, and four recessive genes, *bc-u*, *bc-1*, *bc-2*, and *bc-3*; two of the recessive genes, *bc-1* and *bc-2* have two alleles each, which results in six resistance alleles expressed against BCMV (Drijfhout, 1978; Kelly et al. 1995; Hart and Griffiths 2013). The dominant *I* gene confers extreme resistance or immunity against all strains of BCMV when the temperature stays below 30°C, and variable types of local and systemic necrosis when temperature exceeds 30°C (Ali et al. 1950; Collmer et al. 2000). The mode of action of the *I* gene was identified as hypersensitive resistance (HR), or induced cell death (Collmer et al. 2000; Cadle-Davidson et al. 2005). Of the six recessive resistance alleles, *bc-u* was found to be a helper allele, not conferring resistance by itself but necessary for the expression of BCMV resistance by other five alleles, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3* (Drijfhout, 1978; Kelly et al. 1995). Another of the recessive alleles, *bc-3*, is capable of protecting common bean against almost all strains of BCMV when present together with the *bc-u* helper (Drijfhout, 1978; Kelly et al. 1995; Naderpour et al. 2011; Feng et al. 2015), and was later identified as the *eIF4E* allele carrying a mutated eukaryotic translation initiation factor gene (Naderpour et al. 2010; Hart and Griffith, 2013). The mechanism of the resistance conferred by *bc-3* to BCMV and other potyviruses is related to the block of replication of BCMV due to the impaired interaction between eIF4E protein and the VPg protein covalently linked to the 5'terminus of a potyvirus genome (Naderpour et al. 2010; Hart and Griffith, 2013).

The other four alleles, *bc-1*, *bc-1*^{2}, *bc-2*, and *bc-2*^{2}, confer resistance to BCMV that is highly strain-specific (Drijfhout 1978; Kelly et al. 1995). These four resistance alleles, *bc-1*, *bc-1*^{2}, *bc-2*, and *bc-2*^{2}, are primarily responsible for the large number of pathogroups (PGs), numbered from I to VIII, and defined in the BCMV complex based on resistance or susceptibility in a panel of 11-12 differential

common bean cultivars carrying resistance genes in various combinations (Drijfhout 1978; Drijfhout and Morales 2005; Feng et al. 2015). Based on susceptibility to different pathogroups/strains of BCMV, common bean cultivars were grouped into host groups (HGs) numbered 0 to 11 carrying different combinations of the BCMV resistance alleles (Drijfhout and Morales, 2005). The mode of action of *bc-1* and *bc-2* alleles conferring recessive resistance to BCMV is unknown, although *bc-1* alleles were found involved in restriction of the systemic spread of a related potyvirus, *Bean common mosaic necrosis virus* (BCMNV) (Feng et al. 2017).

Hence the overall goal of this research project was to characterize biologically and molecularly several field isolates of BCMV, and to try to identify the mode of action of the *bc-1* and *bc-2* alleles conferring resistance to BCMV in common bean.

CHAPTER 2: RECESSIVE RESISTANCE TO *BEAN COMMON MOSAIC VIRUS* CONFERRED BY THE *BC-1* AND *BC-2* GENES IN COMMON BEAN *(PHASEOLUS VULGARIS*) AFFECTS LONG-DISTANCE MOVEMENT OF THE VIRUS[1]

Introduction

In the course of a systematic biological and molecular characterization of several field-collected isolates of BCMV, we encountered inconsistencies in pathogroup assignments that varied from experiment to experiment. Initial suspicions that field isolates may represent mixtures of BCMV isolates from different pathogroups, were gradually disproved through back inoculations of progenies picked up from different bean indicators – the same inconsistencies were observed for the same isolate maintained on different bean cultivars. These inconsistencies were expressed as occasional presence of BCMV in upper, asymptomatic non-inoculated leaves, randomly tested by ELISA. After multiple unsuccessful attempts to separate possible mixed infections using differential bean cultivars and back inoculations, an alternative hypothesis was formulated, that 1) some field isolates of BCMV have the ability to overcome *bc-1* and *bc-2* alleles; and 2) these isolates of BCMV may produce an asymptomatic systemic infection in certain common bean cultivars from host groups (HGs) 2, 3, 4, and 6. To test the ability of these field BCMV isolates to invade systemically upper, non-inoculated leaves in common bean cultivars from HG 2, 3, 4, and 6, we decided to test each leaf of the inoculated plant using a laboratory assay, ELISA, capable of detecting an asymptomatic infection. To address possible modes of action of the recessive resistance genes *bc-1* and *bc-2* in *P. vulgaris* more generally, we subjected a set of eight isolates of BCMV from PGs I, IV, VI, VII, and VIII, to a systematic study in a set of bean differentials expressing different combinations of six resistance alleles

[1] This chapter represents a fragment of the published paper by Feng, Orellana, et al. (2018) Recessive resistance to *Bean common mosaic virus* conferred by the *bc-1* and *bc-2* genes in common bean (*Phaseolus vulgaris* L.) affects long distance movement of the virus. *Phytopathology* **108**: 1011-1018 (http://dx.doi.org/10.1094/PHYTO-01-18-0021-R).

including *bc-u*, *bc-1*, *bc-1²*, *bc-2*, *bc-2²*, and *bc-3*. Two types of virus functions were assessed: replication and cell-to-cell movement in the inoculated leaves, and subsequent long-distance movement of the virus to the upper, noninoculated leaves. Expression of *bc-1* and *bc-2* alleles in common bean cultivars was associated with the lack of or impaired long-distance movement of BCMV.

Materials and methods

Virus sources and maintenance

The origins of BCMV isolates RU1-P, RU1-OR, and 1755a were described previously (Feng et al., 2014a,b, 2015). BCMV isolate 3915 was found in a field sample 91-3915 collected in Willamette Valley, OR, in 2015. BCMV isolates 3PF, RU1-CA, and PG1 were collected in 2015 near Davis, CA, by Dr. P. Guzman as field samples from heirloom cultivars of common bean. Isolate 313615 was found in 2013, in a sample of common bean exhibiting mosaic received from a common bean germplasm collection of the USDA-ARS Plant Germplasm Unit, Pullman, WA, provided by Julie Thayer. BCMV isolate Viva2 was collected from a common bean plant exhibiting mosaic, at the VIVA farm near La Conner, WA in 2016. All virus isolates were propagated in the bean cultivar ‘Dubbele Witte’ using mechanical inoculation and maintained under greenhouse conditions as described previously (Feng et al. 2014a).

Biological and serological characterization

The biological characterization of BCMV isolates 3915, 3PF, Viva2, PG1, 313615, and RU1-CA on a set of eleven bean differentials (Drijfhout, 1978) was performed as described previously for BCMV typing (see Table 2.1; Feng et al., 2014a, b). Later, an expanded set of experiments on a sub-group of seven bean cultivars lacking the *I* gene, and carrying different recessive resistance gene

combinations was added to assess the replication, cell-to-cell, and long-distance movement of field isolates under study, in comparison with our control BCMV isolates RU1-P, RU1-OR, and 1755a in these same seven cultivars (see Table 2.2). In the latter experiment, all seven common bean lines were inoculated with each isolate (two or three plants per cultivar), and plants were placed in a climate-controlled growth room with standard summer-time growth conditions (16-hr day photoperiod and daytime/nighttime temperatures of 25/16ºC). Symptoms were recorded at weekly intervals, for 5 weeks post-inoculation (wpi). For each cultivar, inoculated leaves were collected and tested at 2 weeks post-inoculation (wpi); every upper, noninoculated leaf was collected for a triple-antibody sandwich (TAS) ELISA test at 5 wpi. To test the virus presence in leaf tissue, 0.3-0.5-g pieces were cut from each leaf, ground in meshed extraction bags (Bioreba AG, Reinach, Switzerland) using the ELISA extraction buffer (1:10 ratio, w/v), and 100-µl aliquots of the leaf extracts were loaded onto pre-coated ELISA plates as described (Feng et al., 2014b). Triple-antibody sandwich (TAS) ELISA tests using BCMVspecific, polyclonal antibodies produced in two different species, rabbit (coating antibody) and guinea pig (detecting antibody), from the laboratory collection were performed as described previously (Feng et al., 2014b).

Cloning strategy, sequencing, and sequence analysis

The whole-genome cloning for BCMV isolates 3915, 3PF, 313615, PG1, and Viva2 included initial amplification of overlapping RT-PCR fragments as described previously (Feng et al., 2014b). Eight pairs of primers were used, designed based on the aligned BCMV sequences available in the GenBank database (see Table 2.1). RU1-CA genome was amplified by RT-PCR using RU1-P specific primers (Feng et al., 2014b). The overlapping PCR products were cloned into the AT-vector T-Easy (Promega, Madison, WI) and submitted for sequencing to GENEWIZ (South Plainfield, NJ) as described (Feng et al., 2014b). The 5'-terminal sequences for all six isolates were amplified using the RACE Kit (Roche, Indianapolis,IN), and

subsequently cloned and sequenced the same way as the rest of the genome. The complete viral genomes assembled using SeqMan (DNASTAR, Madison, WI) had been deposited in the GenBank database under the accession numbers MH024842 (BCMV-3915),
MH024841 (BCMV-3PF), MH024840 (BCMV-313615), MH024838 (BCMV-PG1), MH024839 (BCMV-Viva2), and MH024843 (BCMV-RU1-CA). All sequences were initially analyzed using the BLASTn 2.2.17 (Altschul et al., 1997) tool available at the National Center for Biotechnology Information (NCBI). Open reading frames (ORFs) were identified using the ORF Finder program available at the National Center for Biotechnology Information (NCBI). Complete sequences of BCMV isolates were aligned using ClustalX Ver. 2.0 (Conway Institute, UCD, Dublin). Further analysis was conducted with the Recombination Detection Program v.4.16 (RDP4) (Martin et al., 2005).

Results

Variable levels of resistance to different BCMV isolates conferred by recessive resistant genes in common bean cultivars

When screened on the eleven bean differentials, all tested BCMV isolates induced typical mosaic, mottle, raised dark green blistering, leaf deformation, and often growth retardation in susceptible bean cultivars. Based on the pathogenicity profiles exhibited on bean differentials, isolate 3915 was classified as belonging to PG-IV, RU1-CA was classified as belonging to PGVI, while four remaining isolates were typed as belonging to PG-I (Table 2.1). However, in the course of biological typing of some of these novel, field BCMV isolates inconsistencies were noted, in particular, occasional presence of the BCMV isolates in upper, non-inoculated leaves detected by ELISA in randomly picked leaves that exhibited no symptoms.

For instance, isolate 3PF was occasionally found present in randomly collected samples from non-inoculated leaves of cultivars ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’, detected by ELISA, while the whole plants remained asymptomatic even at 8 wpi. The pathogenicity test on differential lines was repeated multiple times. Isolate 3PF replicating in upper, asymptomatic leaves of cultivars ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’ was also used as inoculum for re-inoculations of bean differentials and found to exhibit the same profile: ‘Dubbele Witte’ and ‘Stringless Green Refugee’ were always found infected systemically and showed typical symptoms induced by BCMV-3PF infection, while ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’ never exhibited any sign of infection based on visual symptom observations, however, virus could be detected in some randomly collected, but not all upper non-inoculated leaves by ELISA test (see Table 2.2). This suggested the resistance genes incorporated in these cultivars were ‘leaky’ and these cultivars were only partially resistant to BCMV-3PF: virus was able to move systemically to a substantial proportion of upper non-inoculated leaves while the rest of non-inoculated leaves remained uninfected. Additional experiments including seven bean differentials were performed to study variable levels of resistance conferred by recessive genes to novel and control BCMV isolates and assess replication, cell-to-cell, and systemic movement of BCMV isolates in these cultivars. In these additional experiments, 154 plants were inoculated and tested, the number of leaf samples harvested from each plant varied from 23 to 138 and was cultivar and inoculum dependent (Table 2.3). In total, 308 inoculated leaves were tested at 2 wpi, and 6,492 noninoculated leaves were tested at 5 wpi by TAS-ELISA (Table 2.3). RU1-P and RU1-CA were both classified as belonging to PG-VI, and whole genomes of these two isolates were found to share 99% identities to each other (see below). Due to the shortage of space and labor, only RU1-P was subjected to this comprehensive analysis.

When screened on the seven bean differentials lacking the *I* gene, the biological responses induced by five new and three control BCMV isolates in

common bean cultivars carrying different combinations of recessive resistance genes were recorded as symptomatic (typical mosaic and leaf deformations) or asymptomatic (Table 2.2). Based on the visual symptoms alone observed on differentials, isolates 3PF, 313615, Viva2, and PG1 were found able to systemically infect cultivars ‘Dubbele Witte’ (HG-0) and ‘Stringless Green Refugee’ (HG 1), while isolate 3915 infected cultivars ‘Dubbele Witte’ (HG-0), ‘Stringless Green Refugee’ (HG-1), ‘Redlands Greenleaf C’ (HG-2) and ‘Redlands Greenleaf B’ (HG-3) systemically. Control isolates RU1-P, RU1-OR, and 1755a showed the expected susceptibility profiles on the differential lines based on their pathotypes, VI, VII, and VIII, respectively (Table 2.2). The TAS-ELISA detection method confirmed presence of the virus in every single leaf of infected cultivars ‘Dubbele Witte’, ‘Stringless Green Refugee’, ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’ and ‘Sanilac’ (inoculated leaves at 2 wpi and upper non-inoculated leaves at 5 wpi) exhibiting symptoms (Table 2.2, Fig. 2.1). Cultivar ‘UI 35’ inoculated with the isolate RU1-OR exhibited 88% average infection rate among all the upper, non-inoculated leaves at 5 wpi, i.e. 88% of upper, non-inoculated leaves exhibited symptoms and tested positive for BCMV by TAS-ELISA, with 12% of these same non-inoculated leaves being asymptomatic and BCMV negative. These cultivars were marked as susceptible to the corresponding isolates.

All inoculated and upper non-inoculated leaves from bean differentials that did not exhibit visible systemic symptoms were also collected and tested by ELISA for the presence of BCMV the same way (Fig. 2.1). In cultivar ‘IVT7214’ (HG-7), no symptoms were observed and no virus was detected by ELISA test in either inoculated or upper, non-inoculated leaves for all BCMV isolates except 1755a. Consequently, cultivar ‘IVT7214’ was assumed to be immune to all tested BCMV isolates except 1755a. Interestingly, at 2 wpi, all the BCMV isolates were found replicating in all inoculated primary leaves of cultivars ‘Redlands Greenleaf C’ (HG-2), ‘Redlands Greenleaf B’ (HG-3), ‘Sanilac’ (HG-4), and ‘UI 35’ (HG-6), as determined by ELISA, apparently to the same level as the virus infecting susceptible differentials ‘Dubbele Witte’ and ‘Stringless Green Refugee’ (Fig. 2.1). The average

infection rate of upper non-inoculated leaves determined at 5 wpi differed depending of the tested isolate and cultivar combination.

Assessment of the virus replication, cell-to-cell, and long-distance movement in common bean differentials carrying recessive resistance genes

ELISA testing of every single primary inoculated and upper non-inoculated leaf of bean differentials inoculated with the eight BCMV under study was summarized in Table 2.2 and Figure 2.1. In cultivars 'Dubbele Witte' and 'Stringless Green Refugee', with no resistance gene incorporated ('Dubbele Witte') or with only *bc-u* present ('Stringless Green Refugee'), all eight BCMV isolates had no difficulties to replicate or move in both inoculated and upper noninoculated leaves (100% systemic infection). This implied virus could replicate and move freely cell-to-cell in inoculated leaves, and spread systemically through the entire plant when no resistance gene was present or when only *bc-u* was present.

In cultivars 'Redlands Greenleaf C' and 'Redlands Greenleaf B', *bc-1* or *bc-1²* alleles conferred resistance or partial resistance to BCMV isolates 3PF, Viva2, 313615, PG1, and 1755a (Fig. 2.1, A-D, H). In cultivar 'Sanilac', *bc-2* allele conferred resistance or partial resistance to 3PF, Viva2, 313615, PG1, 3915, and RU1-OR (Fig. 2.1, A-E, G). In all cases, virus replication and cell-to-cell movement in inoculated leaves were not affected (Fig. 2.1,Table 2.3). The proportion of infected leaves among all the upper, non-inoculated leaves was dependent on BCMV isolate and bean cultivar: for 'Redlands Greenleaf C' and 'Redlands Greenleaf B' carrying *bc-1* or *bc-1²* allele, the average infection rate ranged from 0 (complete resistance) to 46% (partial resistance), while for 'Sanilac' bearing *bc-2* allele, the rate ranged from 1 (almost complete resistance) to 67% (partial resistance) (Fig. 2.1). This suggested *bc-1* and *bc-2* genes may not affect virus replication and cell-to-cell movement in the inoculated leaves, but they may play an important role in restricting virus long-distance movement in the plant, preventing or impairing systemic spread of the infection.

In cultivar ‘UI 35’, where both *bc-1²* and *bc-2²* allele were present, they apparently conferred complete resistance or partial resistance to all the tested isolates except RU1-OR (Fig. 2.1). Again, the replication and cell-to-cell movement in inoculated leaves were not affected for any of the eight tested BCMV isolates, which was consistent with the profile exhibited in differentials bearing individual *bc-1* or *bc-2* allele. The infection rate of upper, non-inoculated leaves of ‘UI 35’ varied from 0 (complete resistance) to 88% (very weak partial resistance) (Fig.2.1). For most of the isolates, resistance conferred by two recessive genes (*bc-1* and *bc-2*) to BCMV isolates was more effective than that conferred by a single recessive gene (*bc-1* or *bc-2*) (Fig. 2.1).

Whole genome sequencing and sequence analysis

The whole genomes of BCMV isolates 3915, 3PF, Viva2, 313615, and PG1 were cloned and sequenced, using the approach described previously (Feng et al., 2014b). Upon sequence assembly, all five isolates were found to be 10,053-nt long, excluding the poly (A). Based on conceptual translation, all genomes encoded a single polyprotein of the same size of 3,222 aa. The whole genome of BCMV isolate RU1-CA was also cloned and sequenced as described. Upon sequence assembly, RU1-CA was found to be 10,001-nt long, excluding the poly (A). Based on conceptual translation, RU1-CA genome encoded a single polyprotein of 3,202 aa. The coding region for RU1-CA spanned nt 141-9749, total 9609 nts; the coding regions for Viva2, 3PF, 313615, and PG1 all spanned nt 131-9799, and the coding region for 3915 nt 132-9800, total 9669 nts for all five of them. Hence, the the 5’-untranslated region (UTR) of the RU1 strain was slightly (10-nt) longer than for US1/NL1 strains, but the coding region in US1/NL1 types was 60-nt longer than in RU1 type. The 3’-UTR of the RU1 genome was 2-nt shorter than the 3’-UTR of the US-NL1 genomes. These slight differences between sizes of coding and non-coding regions accounted for the observed 20-aa shorter polyprotein of the RU1 strain relative to only 52-nt shorter genome.

The sequences of all six isolates were compared to the known BCMV genomes using the BLASTn 2.2.17 tool (Altschul et al., 1997), and the 3PF and Viva2 sequences were 99% identical to the BCMV strain 2 Iowa sequence (GenBank accession number KU896809), and also 99% identical to the BCMV-NY15p sequence (PG-V; KT175568). Sequences for the isolates 313615 and PG1 shared the closest identities to the NL1 [95% and 92% identical to NL1 (PG-I, AY112735), respectively]. The whole genome sequence of the isolate 3915 was 96% identical to the BCMV isolate NL1 (PG-I; AY112735) and 95% identical to BCMV strain 2 Iowa sequence (KU896809). The whole genome of RU1-CA shared 99% identities to RU1-D (PG-VI; GQ219793) and RU1-P (PG-VI; KF919300).

The whole genomes for NL1 (PG-I; AY112735), 1755a (PG-VIII; KT175570), and 3915 (PG-IV; MH024842), together with PG1 (PG-I; MH024838), Viva 2 (PG-I; MH024839), 313615 (PG-I; MH024840), and 3PF (PG-I; MH024841) were aligned using CLUSTALX and further analysis was conducted with the RDP4 program package. Fig.2.2 (A) shows the comparison of the six sequences using the manual distance plot analysis, with the full-length NL1 sequence used as reference. As can be seen from Fig. 2.2 (A), most of the sequence diversity between BCMV isolates studied was concentrated in two areas: the P1/HC-Pro area, nt 1-2,577, and the NIa/Nib area, nt 5,955-9,028. When the same sequences were analyzed against isolate 1755a as a reference, the area of divergence potentially affecting BCMV isolates' pathogenicity in a *bc-2* genotype (cv. 'Sanilac'), was found in the 5'-terminal region spanning P1, HC-pro and P3 cistron, nucleotides 1 to 3,829 (Fig. 2.2, B).

Discussion

In the past, with a limited set of laboratory tools available to confirm infection, conclusions on susceptibility or resistance to a virus were based on symptom observations, and on back inoculations to indicator plants. The classical study of the genetics of resistance to BCMV in common bean was conducted in the early to mid-1970s, and relied only on visual symptom observations and back inoculations to a

susceptible host (Drijfhout 1978). At that time, a closely related potyvirus, BCMNV was considered a strain of BCMV, and hence treated accordingly, being listed as a set of NL-3, NL-5, and NL-8 strains of BCMV (Drijfhout 1978). Later, based on serological and molecular evidence, BCMNV was recognized as a distinct virus species (McKern et al. 1992; Mink and Silbernagel 1992; Vetten et al. 1992; Berger et al. 1997) exhibiting very limited genetic diversity between these established strains (Strausbaugh et al. 2003; Larsen et al. 2005, 2011; Feng et al. 2017). Recently, the NL-8 strain of BCMNV was demonstrated to have impaired systemic movement in common bean cultivars expressing *bc-1* or *bc-12* resistance alleles, exhibiting incomplete, partial resistance (Feng et al. 2017). Here, we established the same *bc-1* role in the impairment of the systemic movement for BCMV infection, and not only for the two *bc-1* alleles, but also for both *bc-2* and *bc-22* alleles as well. Importantly, we found that the resistance exhibited by all four alleles of the *bc-1* and *bc-2* genes was expressed as an incomplete or partial resistance to many BCMV isolates, identified only when a laboratory detection method was used (Fig. 2.1). It is quite possible that the partial resistance nature of *bc-1* and *bc-2* alleles was not noticed previously (Drijfhout 1978) merely due to the technical limitations of the time.

Dominant *RTM* genes were found to control restricted systemic movement of *Tobacco etch virus* (TEV) in *Arabidopsis thaliana*, conferring resistance against three potyviruses (Mahajan et al., 1998). Similarly, *Wsm1* and *Wsm2*, two dominant resistance genes restricted systemic movement of two potyviruses in wheat (Tatineni et al., 2016). Two recessive resistance genes, *ra* in potato, and *sha3* in *A. thaliana*, were identified to confer resistance to *Potato virus A* and to *Plum pox virus*, respectively, through restriction of systemic spread of the virus (Hamalainen et al., 2000; Pagny et al., 2012). Recently, two alleles of the recessive *bc-1* resistance gene were found to affect systemic spread of BCMNV in common bean (Feng et al. 2017). The exact nature and mechanism of expression of these resistance genes were unknown. But a recessive eIF4 (iso) 4E translation initiation factor gene was implicated in long distance movement of TEV in *A. thaliana*

(Contreras-Paredes et al., 2013). Here, we demonstrated that *bc-1* alleles were involved in restriction of systemic movement not only for BCMNV (Feng et al. 2017), but also for another potyvirus, BCMV (Fig. 2.1, Table 2.2). The restriction of the systemic movement conferred by the *bc-2* alleles may be more specific to BCMV (Fig. 2.1, Table 2.2), although effects on BCMNV may be difficult to assess due to the low genetic and phenotypic diversity of the currently known BCMNV isolates (Feng et al. 2017).

Systemic spread of potyviruses was found to be controlled by three different cistrons, capsid protein (CP) (Carbonell et al., 2013; Decroocq et al., 2009; Desbiez et al., 2014; Dolja et al., 1995; Tatineni et al., 2011), VPg (Hamalainen et al., 2000; Rajamaki and Valkonen, 1999, 2002; Schaad et al., 1997), and 6K2 (Rajamaki and Valkonen, 1999). The most diverse regions in the eight studied BCMV genomes, however, were in the 5'-proximal cistrons, P1, HC-Pro, and P3 (Fig. 2.2). Interestingly, in the BCMNV system, no amino acid substitutions correlating with the observed restriction of the systemic movement of NL-8-like isolates in common bean cultivars expressing *bc-1* alleles, were found in 6K2, VPg, and CP of BCMNV (Feng et al. 2017). The BCMV genetic determinants involved in overcoming the restrictions in systemic movement of the virus need to be defined directly through reverse genetics experimentation.

In breeding for resistance to BCMV, breeders tended to focus on genes conferring broad spectrum, non-strain specific resistance, like *I* and *bc-3* genes (Kelly et al. 1995; Singh and Schwartz 2010). However, wide use of the *I* gene led to potential vulnerability of these common bean genotypes to the BCMNV infection which induced rapid systemic necrosis in *I*-gene bearing cultivars even below 30OC (Ali 1950; Collmer et al. 2000; Feng et al. 2017). On the other hand, a recently described isolate of BCMV, 1755a, was able to overcome the *bc-3* resistance gene (Feng et al. 2015). The data presented here suggest that the use of the two other resistance genes, *bc-1* and *bc-2*, in particular in combinations with each other and with other, non-strain specific resistance genes may provide superior results in breeding for BCMV resistance, restricting or impairing the systemic spread of the

virus in a plant. The data from this research suggest that *bc-1* and *bc-2* alleles provide good to very good resistance to many BCMV strains, especially if deployed in combinations with each other or with other resistance genes.

Table 2.1. Disease and ELISA reactions of bean differentials inoculated with BCMV isolates.[1)]

Bean cultivar[2)]	Resistance genes	HG[3)]	BCMV isolates tested					
			3PF (PG-I)	Viva2 (PG-I)	315615 (PG-I)	PG1 (PG-I)	3915 (PG-IV)	RU1-CA (PG-VI)
'Dubble Witte'	none	0	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**
'SGR'	*i,bc-u*	1	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**
'RGLC'	*i,bc-u,bc-1*	2	-/-	-/-	-/-	-/-	**+/+**	**+/+**
'RGLB'	*i,bc-u,bc-1²*	3	-/-	-/-	-/-	-/-	**+/+**	**+/+**
'Sanilac'	*i,bc-u,bc-2*	4	-/-	-/-	-/-	-/-	-/-	**+/+**
'UI35'	*i,bc-u,bc-1²,bc-2²*	6	-/-	-/-	-/-	-/-	-/-	-/-
'IVT7214'	*i,bc-u,bc-2,bc-3*	7	-/-	-/-	-/-	-/-	-/-	-/-
'Jubila'	*I,bc-1*	9	-/-	-/-	-/-	-/-	-/-	-/-
'Amanda'	*I, bc-1²*	10	-/-	-/-	-/-	-/-	-/-	-/-
'US1006'	*I,bc-u,bc-2²*	11	-/-	-/-	-/-	-/-	-/-	-/-
'IVT7233'	*I,bc-u, bc-1²,bc-2²*	12	-/-	-/-	-/-	-/-	-/-	-/-

[1)] Disease reaction is shown first as a numerator followed by ELISA reaction as a denominator. Three plants were inoculated for each BCMV isolate per experiment and 2-3 leaf samples were collected randomly from upper uninoculated leaves at 4-5 weeks post-inoculation (wpi); numerator: + = symptoms on inoculated plants; - = no symptoms on inoculated plants; denominator: + designates ELISA signal (A405) in an infected plant exceeding healthy control by 10-fold; - designates ELISA signal in an infected plant equivalent to that of a healthy control.

[2)] Abbreviations of common bean cultivars: DW, 'Dubbele Witte'; SGR, 'Stringless Green Refugee'; RGLC, 'Redlands Greenleaf C'; RGLB, 'Redlands Greenleaf B'.

[3)] Host resistance group.

Table 2.2. Symptoms and resistance reaction of select common bean (*P. vulgaris*) differentials inoculated with *Bean common mosaic virus* (BCMV) isolates[1)].

Bean cultivar[2)]	Resistance genes	BCMV isolates tested							
		3PF (PG-I)	Viva 2 (PG-I)	315615 (PG-I)	PG1 (PG-I)	3915 (PG-IV)	RU1P (PG-VI)	RU1-OR (PG-VII)	1755a (PG-VIII)
'DW'	none	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**
'SGR'	*i,bc-u*	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**	**+/S**
'RGLC'	*i,bc-u,bc-1*	-/PR	-/PR	-/PR	-/R	**+/S**	**+/S**	**+/S**	-/R
'RGLB'	*i,bc-u,bc-1*^{2}	-/PR	-/PR	-/PR	-/R	**+/S**	**+/S**	**+/S**	-/PR
'Sanilac'	*i,bc-u,bc-2*	-/PR	-/PR	-/PR	-/PR	-/PR	**+/S**	-/PR	**+/S**
'UI35'	*i,bc-u,bc-1*^{2}*,bc-2*^{2}	-/PR	-/PR	-/PR	-/R	-/R	-/PR	-/PR	-/R
'IVT7214'	*i,bc-u,bc-2,bc-3*	-/IM	-/IM	-/IM	-/IM	-/IM	-/IM	-/IM	**+/S**

[1)] Disease reaction as measured by symptom expression and TAS-ELISA assessment of BCMV infection of both primary inoculated and upper uninoculated leaves in different genotypes. Two or three plants were inoculated for each BCMV isolate per experiment, primary inoculated leaves were tested at 2 weeks post-inoculation (wpi) and upper uninoculated leaves were tested at 5 wpi; numerator: + = symptoms on inoculated plants; - = no symptoms on inoculated plants; denominator: S, susceptible, designates virus was able to replicate and spread to the entire plant; IM, immune, designates no virus was found in either primary inoculated leaves or upper noninoculated leaves; R, resistant, designates virus found replicating and moving normally in primary inoculated leaves but unable to move beyond the inoculated leaves; PR, partially resistant, designates viruses able to replicate and move from cell-to-cell in primary inoculated leaves, but their long-distance movement ability was impaired and infection could only be found in a few upper non-inoculated leaves.

[2)] Abbreviations of common bean cultivars: DW, 'Dubbele Witte'; SGR, 'Stringless Green Refugee'; RGLC, 'Redlands Greenleaf C'; RGLB, 'Redlands Greenleaf B'.

Figure 2.1. A-H.

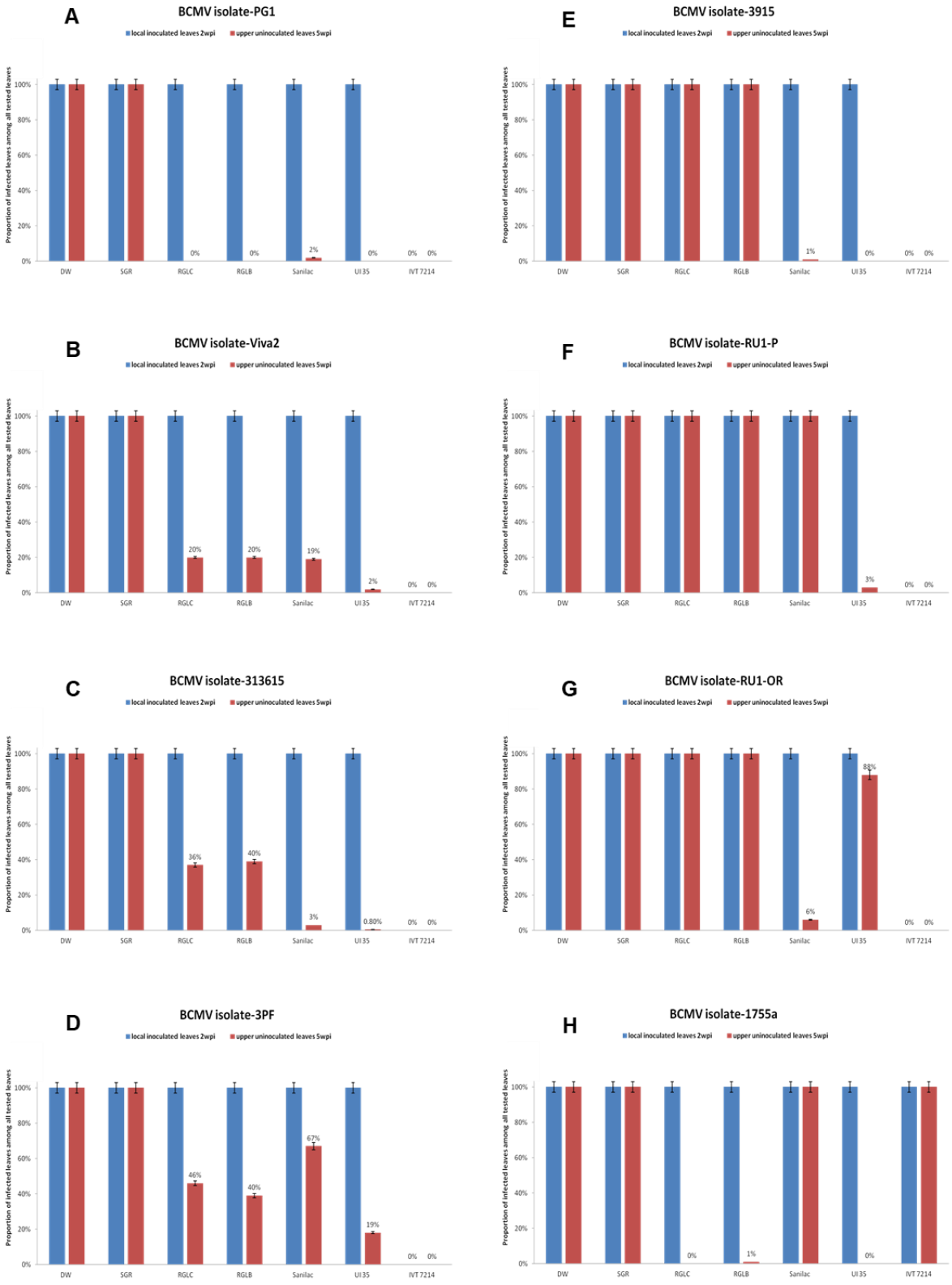
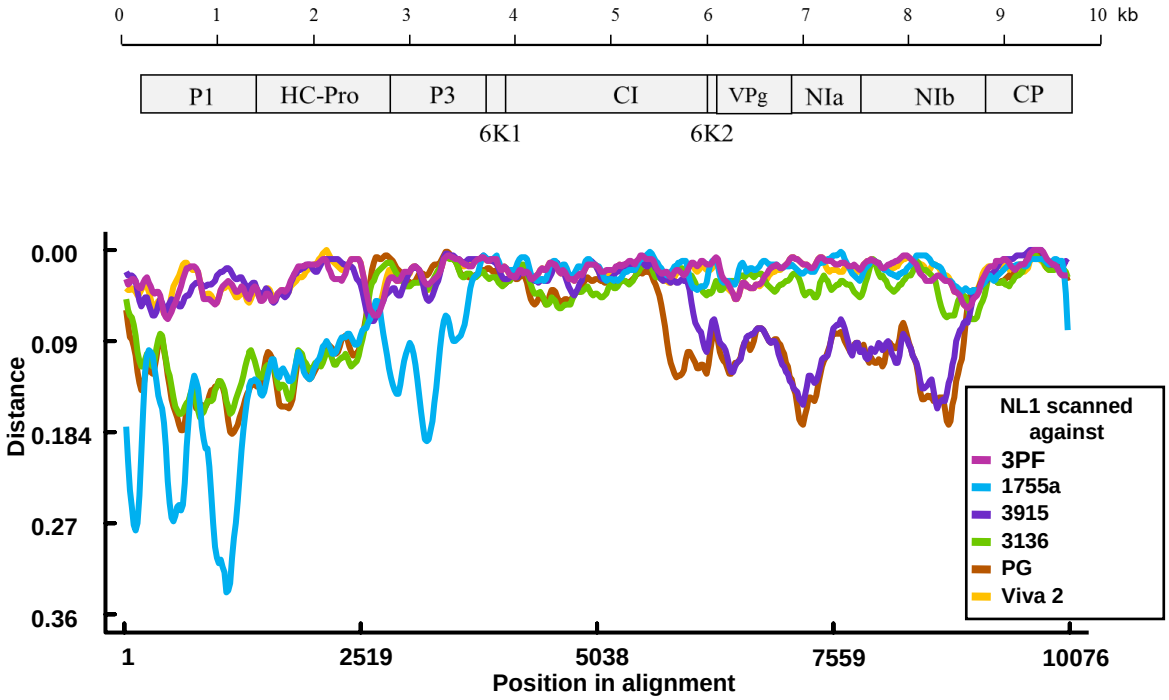

A
BCMV isolate-PG1
local inoculated leaves 2wpi
upper uninoculated leaves 5wpi
Proportion of infected leaves among all tested leaves
100%
80%
60%
40%
20%
0%
0%
0%
2%
0%
0% 0%
DW
SGR
RGLC
RGLB
Sanilac
UI 35
IVT 7214
B
BCMV isolate-Viva2
20%
20%
19%
2%
0% 0%
C
BCMV isolate-313615
36%
40%
3%
0.80%
0% 0%
D
BCMV isolate-3PF
46%
40%
67%
19%
0% 0%
E
BCMV isolate-3915
1%
0%
0% 0%
F
BCMV isolate-RU1-P
3%
0% 0%
G
BCMV isolate-RU1-OR
6%
88%
0% 0%
H
BCMV isolate-1755a
0%
1%
0%
0% 0%

Figure 2.1. **A-H**. Eight *Bean common mosaic virus* (BCMV) isolates were screened in seven common bean differentials carrying different recessive resistance genes.The upper non-inoculated leaves were tested at 5 weeks post-inoculation (wpi) by TAS-ELISA for BCMV infection. The proportion of infected leaves per plant among all the tested leaves was calculated to assess cell-to-cell and longdistance movement ability of BCMV isolates in different common bean genotypes. Vertical bars represent the average inoculated and non-inoculated leaf infection rate for two or three plants of the same cultivar analyzed by TAS-ELISA in the same experiment.

Abbreviations of common bean cultivars: DW, ‘Dubbele Witte’; SGR, ‘Stringless Green Refugee’; RGLC, ‘Redlands Greenleaf C’; RGLB, ‘Redlands Greenleaf B’

Figure 2.2 Recombination analysis of the 5 studied *Bean common mosaic virus* (BCMV) isolates, 3915, 3PF, Viva2, 313615, and PG1, in comparison to the control BCMV isolates 1755a and NL1

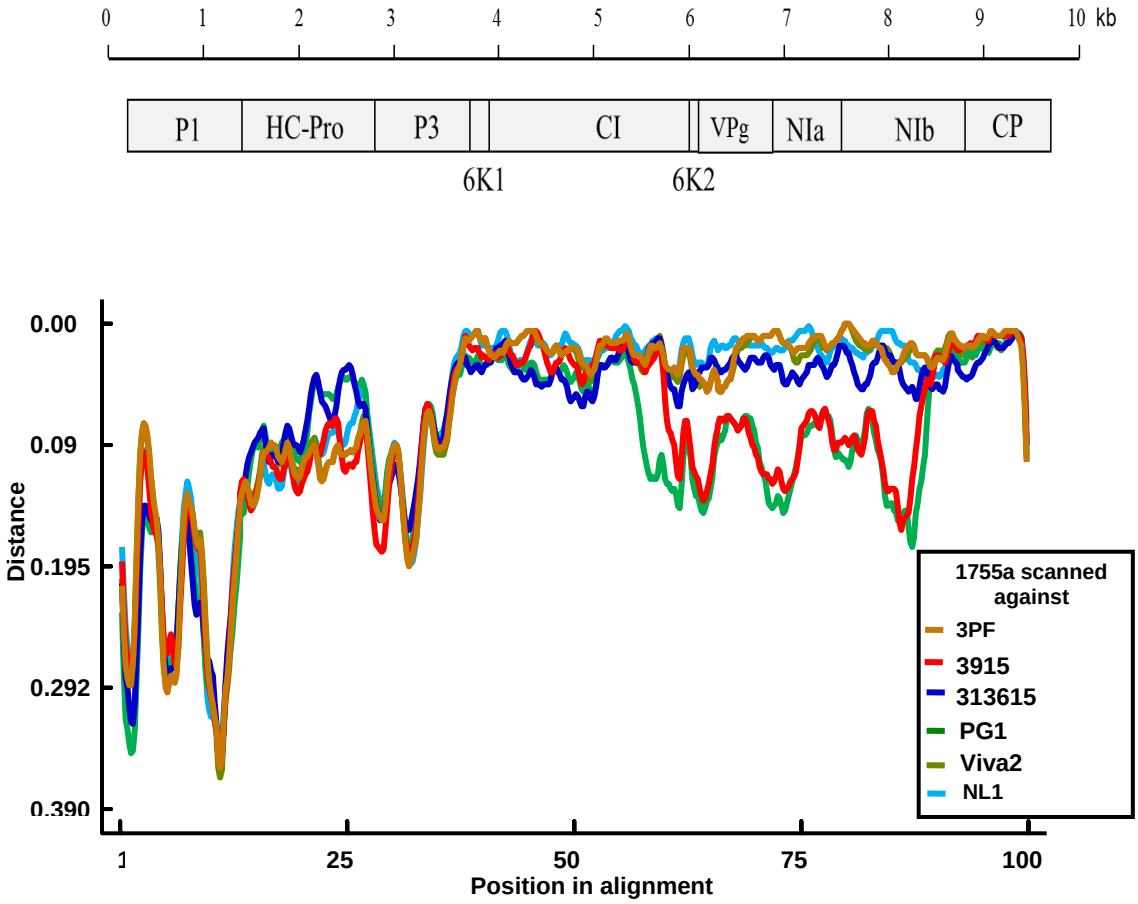
Figure 2.2.A



(A) Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates 3915, 3PF, Viva2, 313615, PG1, 1755a, and NL1. Sequence of isolate NL1 (PG-I; accession number AY112735) was used as the reference.

X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence, calculated using Kimura model (Kimura, 1980)

Figure 2.2.B



(B) Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates 3915, 3PF, Viva2, 313615, PG1, 1755a, and NL1. Sequence of isolate 1755a (PG-VIII; accession number KT175570) was used as the reference.

X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence, calculated using Kimura model (Kimura, 1980).

Table 2.3. ELISA testing results for individual primary inoculated and upper uninoculated leaves of select common bean (*P. vulgaris*) differential cultivars inoculated with eight *Bean common mosaic virus* (BCMV) isolates[1].

BCMV - 3PF				
Bean cultivars[2]	Tested plant number	Infected/total inoculated leaf number (2wpi)	Infected/total systemic leaf number (5wpi)	Infected upper uninoculated leaf % (5wpi)
DW	2	4/4	75/75	100%
SGR	2	4/4	63/63	100%
RGLC	3	6/6	46/99	46%
RGLB	3	6/6	27/68	40%
Sanilac	3	6/6	66/99	67%
UI 35	3	6/6	14/75	19%
IVT7214	3	0/6	0/196	0%
BCMV - PG1				
DW	2	4/4	79/79	100%
SGR	2	4/4	74/74	100%
RGLC	3	6/6	0/108	0%
RGLB	3	6/6	0/98	0%
Sanilac	2	4/4	3/136	2%
UI 35	3	6/6	0/179	0%
IVT7214	2	0/4	0/97	0%
BCMV - Viva2				
DW	3	6/6	127/127	100%
SGR	2	4/4	61/61	100%
RGLC	2	4/4	10/51	20%
RGLB	2	4/4	13/65	20%
Sanilac	4	8/8	33/171	19%
UI 35	2	4/4	4/175	2%
IVT7214	3	0/6	0/117	0%
BCMV - 313615				
DW	2	4/4	69/69	100%
SGR	2	4/4	95/95	100%
RGLC	2	4/4	24/66	36%
RGLB	2	4/4	24/60	40%
Sanilac	2	4/4	3/105	3%
UI 35	2	4/4	1/127	0.80%
IVT7214	2	0/4	0/107	0%

BCMV - 1755a				
DW	2	4/4	73/73	100%
SGR	3	6/6	91/91	100%
RGLC	3	6/6	0/92	0%
RGLB	3	6/6	1/101	1%
Sanilac	3	6/6	119/119	100%
UI 35	3	6/6	0/133	0%
IVT7214	3	6/6	139/139	100%
BCMV - RU1-OR				
DW	3	6/6	104/104	100%
SGR	3	6/6	118/118	100%
RGLC	2	4/4	56/56	100%
RGLB	3	6/6	124/124	100%
Sanilac	4	8/8	12/206	6%
UI 35	5	10/10	346/391	88%
IVT7214	3	0/6	0/179	0%
BCMV - RU1-P				
DW	3	6/6	120/120	100%
SGR	3	6/6	57/57	100%
RGLC	3	6/6	83/83	100%
RGLB	3	6/6	97/97	100%
Sanilac	2	4/4	41/41	100%
UI 35	5	10/10	11/347	3%
IVT7214	3	0/6	0/190	0%
BCMV - 3915				
DW	3	6/6	104/104	100%
SGR	3	6/6	84/84	100%
RGLC	3	6/6	88/88	100%
RGLB	3	6/6	102/102	100%
Sanilac	3	6/6	1/109	1%
UI 35	3	6/6	0/143	0%
IVT7214	3	0/6	0/159	0%

[1)] Two or three plants were inoculated for each BCMV isolate per experiment, primary inoculated leaves were tested at 2 weeks post-inoculation (wpi) and upper uninoculated leaves were tested at 5 wpi; numerator: number of BCMV-positive leaves as determined by TAS-ELISA; denominator: total number of BCMV-tested leaves.

[2)] Cultivar abbreviations: DW, ‘Dubbele Witte’; SGR, ‘Stringless Green Refugee’; RGLC, ‘Redlands Greenleaf C’; RGLB, ‘Redlands Greenleaf B’.

Table 2.4. Primers used for cloning the whole BCMV genome

Primer	Position	Sequence
BCMV-1F	116	GGAAAATCATCTGAAATGGC
BCMV-1R	1610	CTGGATACAGCATCTGGC
BCMV-2F	1411	GGTGAATGCACTGGATGAAC
BCMV-2R	2940	CCCATCAGTAGGATATAGGG
BCMV-3F	2616	CAGCGGTTTACATTCTTACAGTG
BCMV-3R	4065	TTCTTCTCCTCATCCACGCTC
BCMV-4F	3767	CCCCCAACCAGTGATGA
BCMV-4R	5222	GTGTTGTGACAGGTAGTCC
BCMV-5F	5078	CAGAGACTTGGAAGGG
BCMV-5R	6400	CCTGATCTCACCGAAC
BCMV-6F	6179	GAACACACATTTGGTG
BCMV-6R	7655	CAAACGCTTCATAGCAAACC
BCMV-7F	7413	CGGTAGAAGGCAATCTC
BCMV-7R	8927	GCACAGATTCTCCGCATCC
BCMV-8F	8750	CTGTGGCTGCTTGAAAGGG
Anchor-8R	3’ end of genome	GACCACGCGTATCGATGTCGAC
Anchor- 1st strand RT	3’ end of genome	GACCACGCGTATCGATGTCGAC$(T)_{17}$A
3915: 5RACE-PCR	247	GTTTCCATGCACACCTCCTG
3PF/ Viva2 5RACE-PCR	294	CGTGCTGAGCATCCTACAG
313615: 5RACE-PCR	221	CCTGGTGGTTTCCACCATCC
PG1: 5RACE-PCR	241	CTTCCTCCTCAAGCCTG

CHAPTER 3: A NEW STRAIN OF *BEAN COMMON MOSAIC VIRUS* FROM LIMA BEAN (*PHASEOLUS LUNATUS*): BIOLOGICAL AND MOLECULAR CHARACTERIZATION[2]

Introduction

Bean common mosaic virus (BCMV) exists as a complex of strains that produce varying symptoms on a range of several hosts displaying genetic diversity and even recombinant genomes. BCMV genome sequences can vary between 82% and 94%. Because of lima bean's short history in the United States, BCMV isolates infecting *Phaseolus lunatus* have not yet been characterized. In 2017, two symptomatic lima bean plants exhibiting mosaic, vein banding, and growth retardation were collected in a community garden in Honolulu, HI. BCMV was detected in both plant samples. The two BCMV isolates were initially considered as two different isolates and analyzed separately, being subjected to biological characterization on a panel of 11 differential cultivars of common bean (*P. vulgaris*), expressing different combinations of six resistance alleles including *bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3*.

The overall goal of this project was to determine a potential risk associated with these lima bean isolates of BCMV (named BCMV-A1) for common bean, that is if they can infect and threaten *P. vulgaris*. In the process of the studies, we characterized their biological and molecular properties, and determined that both represent a new strain of BCMV. Approximately at the same time period, we found

[2] This chapter represents a fragment of the published paper by Feng, Orellana, et al. (2019) A new strain of *Bean common mosaic virus* from lima bean (*Phaseolus lunatus*): biological and molecular characterization. *Plant Disease*, published on-line January 11, 2019 (http://dx.doi.org/10.1094/PDIS-08-18-1307-RE).

almost identical isolates of BCMV in common beans grown in Idaho, confirming the potential threat of BCMV-A1 for common bean.

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, 101 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 eleven 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 eleven 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-hr day photoperiod and daytime/nighttime temperatures of 25/16ºC). Symptoms in inoculated and systemic non-inoculated leaves were recorded at weekly intervals,

up to 5 weeks post-inoculation (wpi). For *I* gene containing cultivars including ‘Jubila’, ‘Amanda’, ‘US1006’ and ‘IVT7233’, 2-3 leaf samples were randomly collected from the upper non-inoculated leaves at 5 wpi and tested by TAS-ELISA. For the rest of differential lines lacking *I* gene, and carrying combined recessive alleles, samples from each single inoculated and upper non-inoculated leaves 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 triple-antibody sandwich (TAS) ELISA tests 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 3.1). 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 3.1; Feng et al. 2014b, 2015). The PCR profile consisted of denaturing at 94ºC for 2 min; 35 cycles of 94ºC for 20s, 50- 55ºC for 30s (depending on the melting temperatures of primers used); and 72ºC for 1-1.5 min (depending on the fragment length amplified); followed by a final extension for 10 min at 72ºC. 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 the 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 eleven other BCMV isolates and one BCMNV isolate using ClustralX Ver. 2.0 (Conway Institute, UCD, Dublin). Among twelve BCMV isolates, were two BCMV-PStV isolates, two soybean isolates, two were RU1 type, and another two US1 type BCMV isolates. Additionally, two were BCMV-BlCMV 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 twelve 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) phylogeny trees were generated in MEGA 6 (Tamura et al. 2013). To test the robustness of individual branch, 100 replicates were used to perform bootstrap analysis. BCMNV (HQ229995) was used as an outgroup species. PStV-JX014 (KJ807813), BlCMV (AY575773), RU1-P (KF919300), and US1 (KT175569) sequences were aligned 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 x 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 weeks post-inoculation (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, non- inoculated leaves (Table 3.4). Nevertheless, ELISA testing of the randomly selected upper, non-inoculated 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 3.2). 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. 3.1). Later, leaf yellowing and vein necrosis started to develop at around 3 wpi (18-21 dpi) (Fig. 3.1). In cv. 'Dubbele Witte', the plants were severely stunted as the result of the virus infection (Fig. 3.2); this stunting became very prominent as early as 13 dpi (Fig. 3.2). By 18 dpi, the newly

developed upper non-inoculated leaves exhibited severe systemic vein necrotic and the infected plant wilted and died at around 3 wpi (Fig. 3.2).

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, non-inoculated leaves from each of the inoculated plant 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 3.3). 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 3.3). 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 non-inoculated leaves of the two cultivars where it exhibited systemic symptoms, 'Dubbele Witte', and 'Stringless Green Refugee' (Table 3.2), 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 non-inoculated leaves in three cultivars carrying single *bc-1* or *bc-2* alleles, 'Redlands Greenleaf C', 'Redlands Greenleaf B', and 'Sanilac' (Table 3.2). 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 3.2).

Whole genome sequencing and sequence analysis

The assembled overlapping amplicons of BCMV isolates A1 and A2 were both found to be 9,995-nucleotidet (nt) long, excluding the poly (A). The A1 and A2 sequences were nearly identical (≈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 polyprotein 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, twelve 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. 3.3) 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. 3.3). 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. 3.3). In pair-wise comparisons, BCMV isolates from clade I shared 86% nucleotide sequence identity with clade II and 84-86% sequence identity with clade III. Clades II and III shared 83-85% nucleotide sequence identity.

To visualize the distribution of the genetic diversity along entire genome, the whole genomes for BCMV-A1 and four other known BCMV strains were aligned and subjected to recombination analysis. Fig. 3.4 (A) 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 Fig. 3.4 (A), 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 cv. 'Dubble 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. 3.4, A). The nucleotide sequence for the F17:0298A isolate was re-named 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 twelve BCMV isolates analyzed in Fig. 3.3 was aligned using ClustalX and phylogenies were inferred using BCMNV-TN1 sequence (accession number HQ229995) as outgroup. The relationships between strains of BCMV based on the partial genome analysis of the HC-Pro region (Fig. 3.4, B) were consistent with phylogenies based on whole genome sequence of the same twelve BCMV strains (Fig. 3.3). 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. 3.4, B).

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 to type 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 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 (Larsen et al. 2005; Feng et al. 2015). 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,b, 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 (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 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 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. 3.2), and a less severe systemic necrosis in 'Stringless Green Refugee' (Table 3.2), 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; Drijfhout 1978; Collmer et al. 2000; 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 37-95% of non-inoculated 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 3.2) 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. 3.4, B). 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.

Table 3.1 Primers used for sequencing of *Bean common mosaic virus* (BCMV) isolate A1 and BCMV-ID genomes.

Primer	Position	Sequence
Whole genome sequencing		
SP F1	1	AATTAAAACAACTCATAAAGAC
SP R1	1514	GCCCACACTGTGCATTGTC
SP F2	1351	GGTGAATGCACTGGATGAAC
SP R2	2880	CCCATCAGTAGGATATAGGG
SP F3	2774	GATGCACAACAAAGAATGCG
SP R3	4053	GTTGAAGTAGCTTCCTTGC
SP F4	3685	GTGTTGCAAAGTCACAGGT
SP R4	5333	CCATTTCTGATTCTCTCAGC
SP F5	5157	CAACACAAGGTGTCACTAC
SP R5	6283	CAAAGTTGCACCAGTGAGTG
SP F6	6089	CGCGAGGTGTATGCTGATG
SP R6	7681	GTATATCTCCTCTGGATCTG
SP F7	7399	GGTGACAAAGCATGTAGTG
SP R7	8864	CAGATTCTCCACATCCAAC
SP F8	8628	GTGCAGCAATGATTGAAGC
Anchor-8R	3’ end of genome	GACCACGCGTATCGATGTCGAC
1st strand RT	3’ end of genome	GACCACGCGTATCGATGTCGAC(T)17A
SP 5RACE-PCR	214	CTATGAGCTCTTCGACTTCC
Partial genome sequencing		
BCMV-NL1-1950F	1925	CGGAGGCTGAYCCACTGAAGAC
BCMV-NL1-2700R	2623	CCTGTGGTGAGGGATCCAAAT

Table 3.2. Resistance of bean differentials inoculated with BCMV isolates.[1)]

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,bc-u*	**+/S**	**+/S**	**+/S**	**+/S**
'RGLC'	*i,bc-u,bc-1*	-/PR	**+/S**	**+/S**	-/R
'RGLB'	*i,bc-u,bc-12*	-/PR	**+/S**	**+/S**	-/PR
'Sanilac'	*i,bc-u,bc-2*	-/PR	**+/S**	-/PR	**+/S**
'UI35'	*i,bc-u,bc-12,bc-22*	-/R	-/PR	**+**/PR	-/R
'IVT7214'	*i,bc-u,bc-2,bc-3*	-/IM	-/IM	-/IM	**+/S**

[1)] Disease reaction is measured by symptom observation and TAS-ELISA assessment of BCMV isolates for infection of both local inoculate and upper non-inoculated leaves in different genotypes. Two or three plants were inoculated for each BCMV isolate per an experiment, local inoculated leaves were tested at 3 wpi and upper non-inoculated 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 non-inoculated leaves; R designates viruses were found replicating and moving normally in local inoculated leaves but unable to move beyond the inoculated leave; 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 non-inoculated leaves.

Table 3.3. 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, non-inoculated 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/*bc-u*	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/*bc-u, bc-1*	1	**2/2**	100%	**2/24**	8%	ClSp	NS
	2	**2/2**	100%	**11/30**	37%		
	3	**2/2**	100%	**8/27**	30%		
RGLB/*bc-u, bc-12*	1	**2/2**	100%	**18/32**	56%	ClSp	NS
	2	**2/2**	100%	**12/24**	50%		
	3	**2/2**	100%	**12/25**	48%		
Sanilac/*bc-u, bc-2*	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/*bc-u, bc-12, bc-22*	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/*bc-u, bc-2, bc-3*	1	**0/2**	0%	**0/31**	0%	NS	NS
	2	**0/2**	0%	**0/38**	0%		
	3	**0/2**	0%	**0/33**	0%		

Abbreviations:

a) DW, ‘Dubbele Witte’; SGR, ‘Stringless Green Refugee’; RGLC, ‘Redland Greenleaf C’; RGLB, ‘Redland Greenleaf B’

b) Wpi, weeks post-inoculation

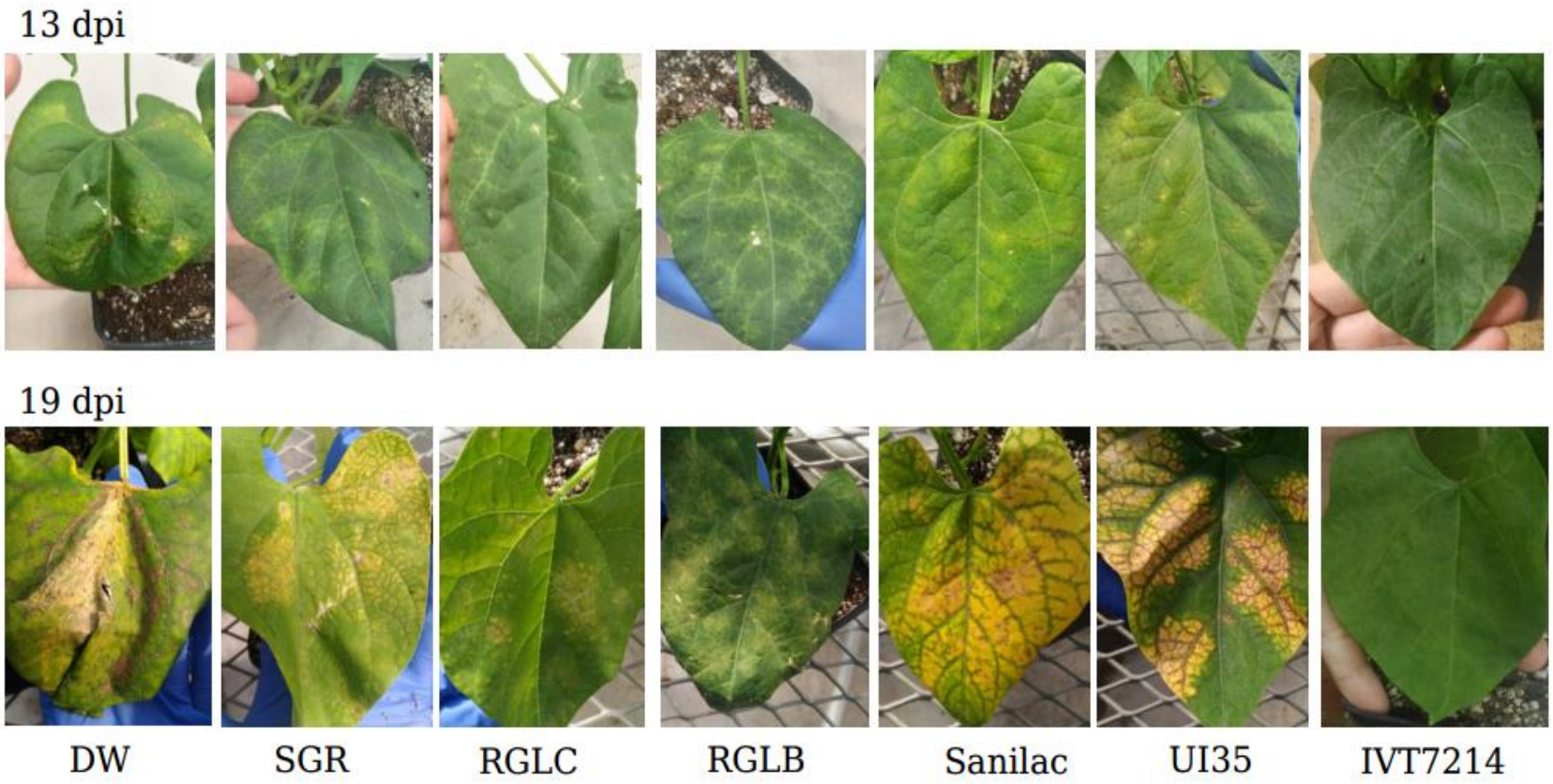
c) IL, inoculated leaf; NIL, upper non-inoculated leaf; NLL, necrotic local lesions; syst. N, systemic necrosis; MM, mild mosaic; VN, vein necrosis; ClSp, chlorotic spots; NS, no symptoms

Table 3.4. Disease and ELISA reactions of bean differentials inoculated with *Bean common mosaic virus* (BCMV) isolates.1) Bean cultivars are 'Dubbele Witte', 'Stringless Green Refugee' (SGR), 'Redlands Greenleaf C' (RGLC), 'Redlands Greenleaf B' (RGLB), 'Sanilac', 'UI35', 'IVT7214', 'Jubila', 'Amanda', 'US1006', and IVT7233'.

Bean cultivar	Resistance genes	BCMV isolate (pathogroup)				
		A1 (PG-I)	A2 (PG-I)	RU1-P (PG-VI)	RU1-OR (PG-VII)	1755a (PG-VIII)
'Dubble Witte'	none	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**
'SGR'	*i,bc-u*	**+/+**	**+/+**	**+/+**	**+/+**	**+/+**
'RGLC'	*i,bc-u,bc-1*	-/-	-/-	**+/+**	**+/+**	-/-
'RGLB'	*i,bc-u,bc-12*	-/-	-/-	**+/+**	**+/+**	-/-
'Sanilac'	*i,bc-u,bc-2*	-/-	-/-	**+/+**	-/-	**+/+**
'UI35'	*i,bc-u,bc-12,bc-22*	-/-	-/-	-/-	**+/+**	-/-
'IVT7214'	*i,bc-u,bc-2,bc-3*	-/-	-/-	-/-	-/-	**+/+**
'Jubila'	*I,bc-1*	-/-	-/-	-/-	-/-	-/-
'Amanda'	*I, bc-12*	-/-	-/-	-/-	-/-	-/-
'US1006'	*I,bc-u,bc-22*	-/-	-/-	-/-	-/-	-/-
'IVT7233'	*I,bc-u, bc-12,bc-22*	-/-	-/-	-/-	-/-	-/-

1) Disease reaction is shown first as a numerator followed by ELISA reaction as a denominator. Three plants were inoculated for each BCMV isolate per an experiment and 2-3 leaf samples were collected randomly from upper uninoculated leaves at 5 wpi; numerator: + = symptoms on inoculated beans; - = no symptoms on inoculated beans; denominator: + designates ELISA signal (A405) in an infected plant exceeding healthy control 10-fold or more; - designates ELISA signal in an infected plant equal to that of a healthy.

Figure 3.1. Symptoms exhibited by the *Bean common mosaic virus* isolate A1 on inoculated leaves of seven (*Phaseolus vulgaris*) cultivars. These are ‘DubbeleWitte’, ‘Stringless Green Refugee’, ‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, ‘Sanilac’, ‘UI35’, and ‘IVT7214’. Symptoms were observed at 13 dpi (upper row), and at 19 dpi (bottom row).



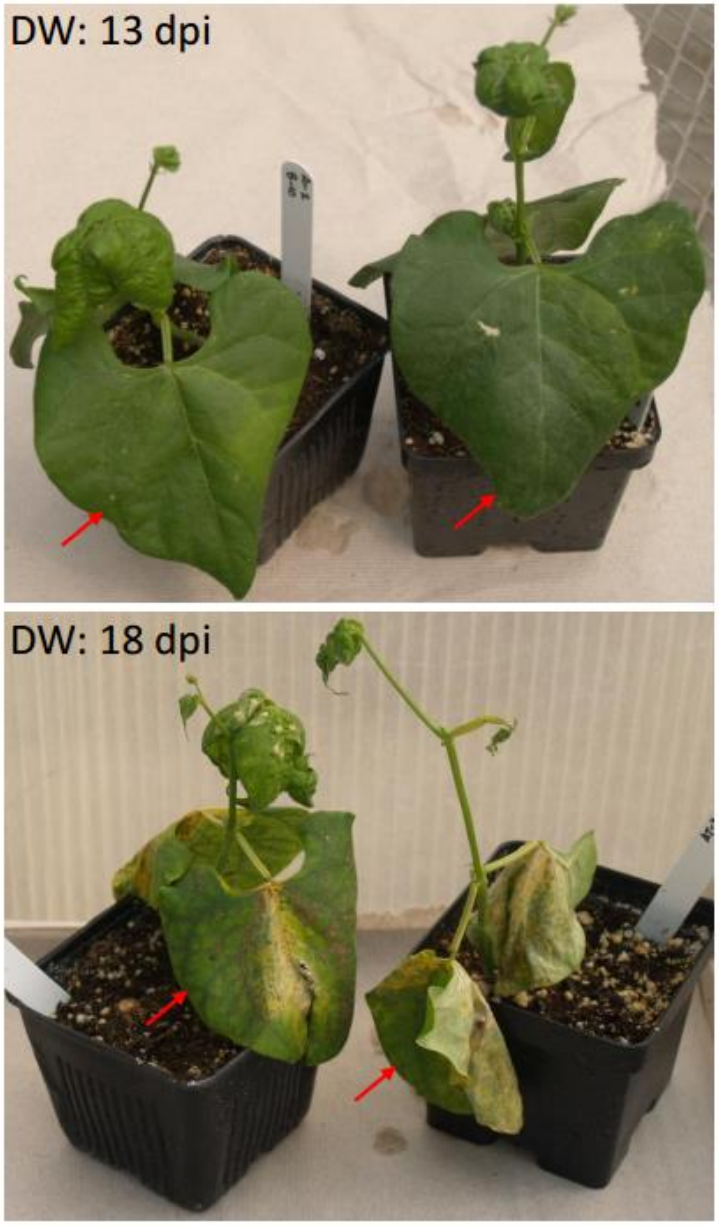


Figure 3.2.Systemic symptoms induced by the *Bean common mosaic virus* (BCMV) isolate A1 in common bean (*Phaseolus vulgaris*) cultivar ‘Dubbele Witte’ (DW) at 13 and 18 days post-inoculation (dpi); arrows point at inoculated leaves.

Figure 3.3. 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. Blue brackets indicate lineages of US1, RU1, BlCMV, 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.

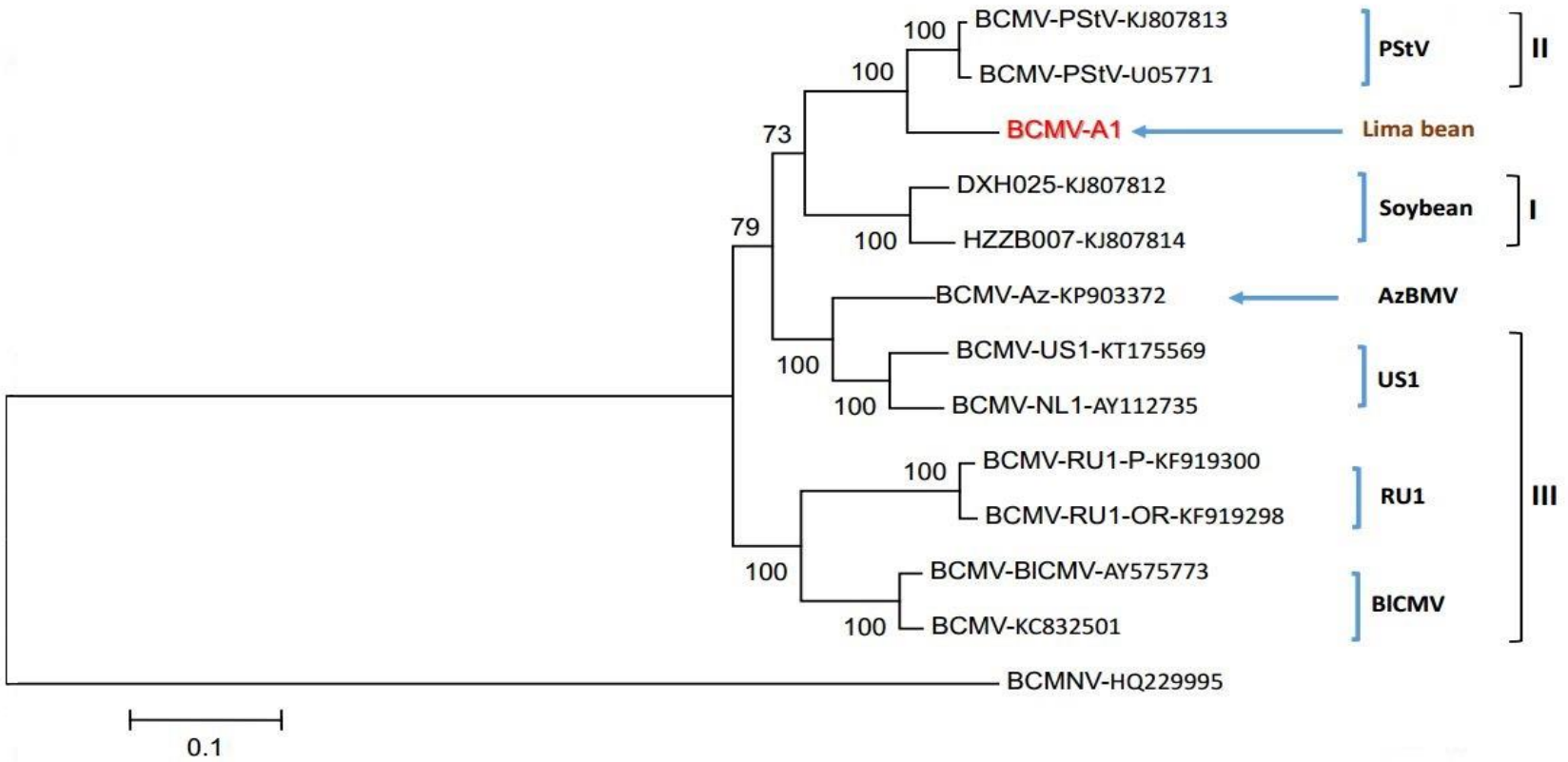


Figure 3.4. (A) Recombination analysis of the whole genome of the *Bean common mosaic virus* (BCMV) isolate A1. Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates from the main BCMV lineages; BCMV-A1 sequence was used as a reference. The green double-arrow indicates the position of the BCMV-ID genome fragment analyzed in panel B.

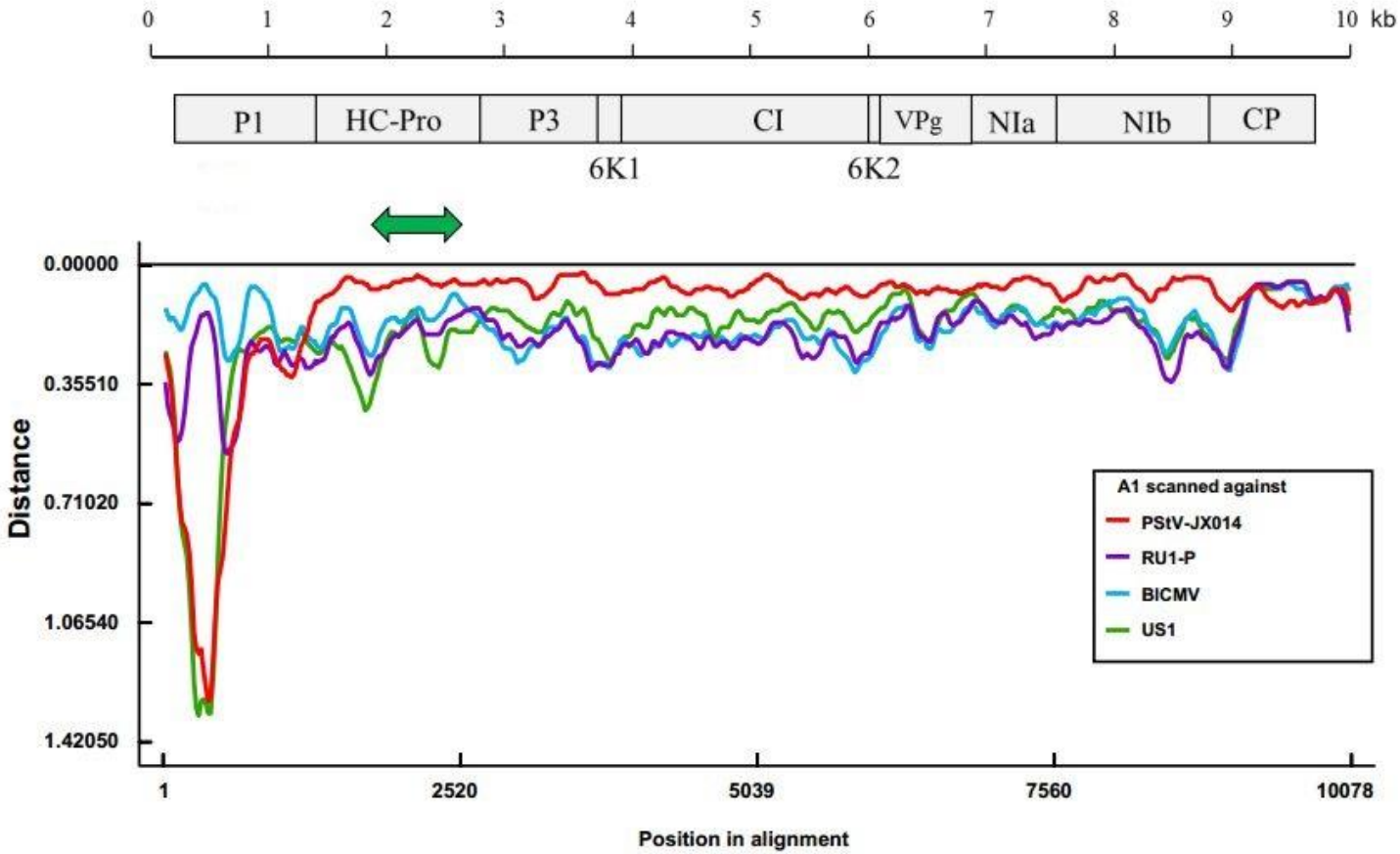
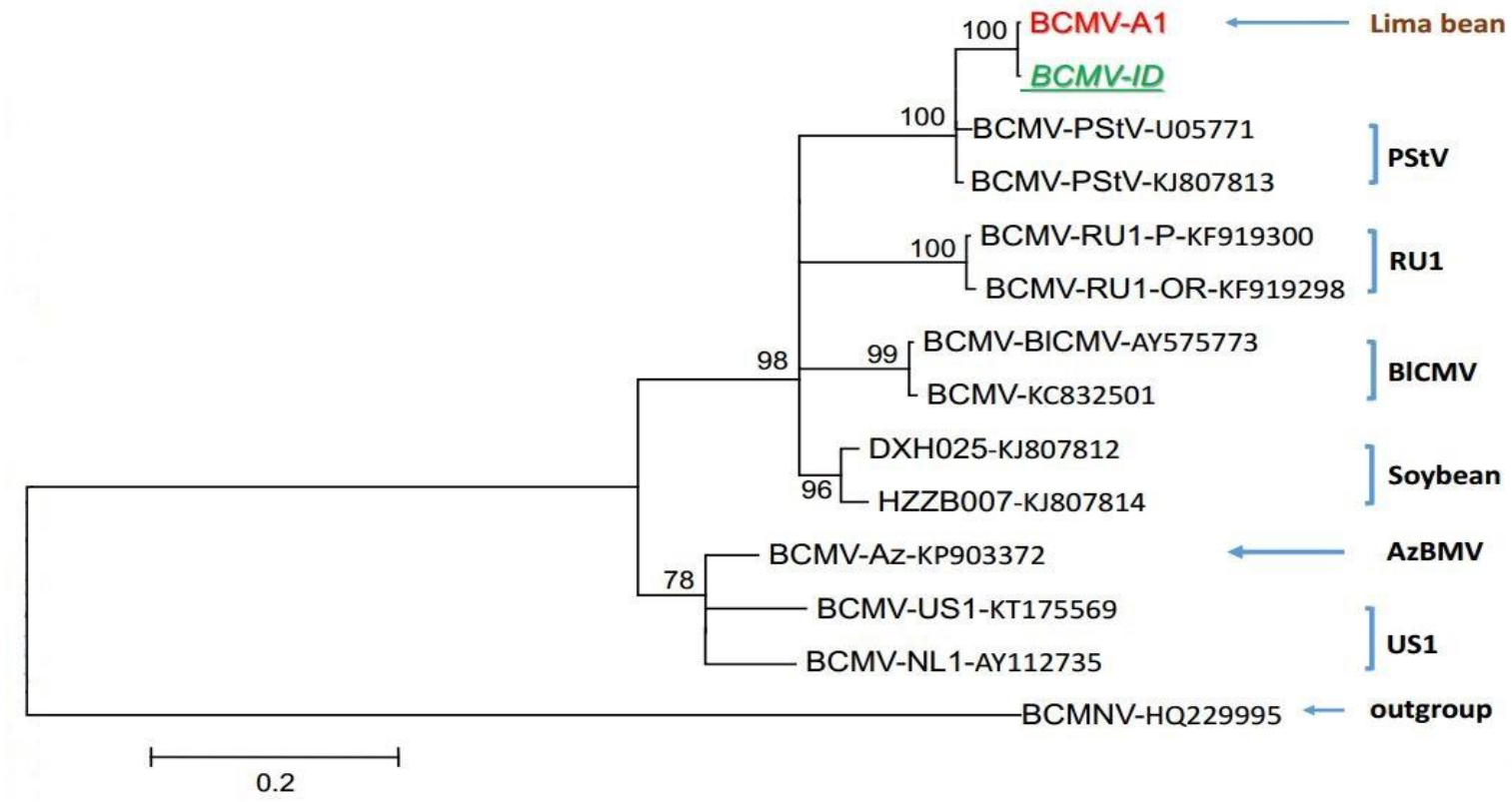


Figure 3.4. (B) Phylogenetic analysis of the partial genome sequence of the BCMV-ID isolate. Maximum-likelihood phylogenetic tree for the genome segment (nt 1973-2683) of select BCMV isolates, plus a partial sequence of the corresponding genome fragment for BCMV-ID. Position of the analyzed genome segment 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. Blue brackets and arrows indicate lineages of US1, RU1, BlCMV, Soybean, and PStV, associated with strain groups shown on Fig. 3.3. *Bean common mosaic necrosis virus* (BCMNV), isolate TN1 sequence was used as an outgroup.



CHAPTER 4: CONCLUSIONS

The overall goal of this research project was to characterize biologically and molecularly several field isolates of BCMV, and to try to identify the mode of action of the *bc-1* and *bc-2* genes conferring recessive resistance to BCMV in common bean.

This study establishes the role of *bc-1* and *bc-2* genes in impairment of the systemic movement of BCMV in common bean (*P. vulgaris*). The resistance conferred by all four alleles of the *bc-1* and *bc-2* genes was expressed as an incomplete or partial resistance to many BCMV isolates, with this incomplete nature identified only when a laboratory detection method was used (Fig. 2.1). This proves that conclusions on susceptibility or resistance to BCMV cannot rely only on symptom observations, laboratory techniques must be use to prove or discard the presence of the virus. Furthermore, this data suggest that *bc-1* and *bc-2* resistance genes may provide superior results in breeding for BCMV resistance restricting or impairing the systemic spread of the virus in a plant if stacked with other resistance genes affecting other stages of the virus life cycle.

Phylogenetically, BCMV isolates can be grouped according to their legume host specificities, with two clades (US1 and RU1) defined in common bean. However, the biological diversity of the BCMV isolates originating from lima bean (*P. lunatus*) has been poorly studied. This study documents BCMV-A1 which is the first BCMV isolate from lima bean subjected to a complete biological and molecular characterization. BCMV-A1 is capable of inducing systemic necrotic reaction in ‘Dubbele Witte’ (Fig. 3.2) and middle systemic necrosis in ‘Stringless Green Refugee’ (Table 3.2). BCMV-A1 can also stablish an asymptomatic infection in some common bean cultivars (‘Redlands Greenleaf C’, ‘Redlands Greenleaf B’, and ‘Sanilac’) suggesting that 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.

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