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An *RNase H-Like* gene complements resistance to *Bean common mosaic necrosis virus* in *Phaseolus vulgaris*

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Abstract

Bean common mosaic virus and *Bean common mosaic necrosis virus* (BCMNV) are related positive-sense RNA *potyviruses* that limit the production of common bean (*Phaseolus vulgaris* L.) worldwide. *Potyviruses* infect other legume species, such as *Glycine max*, which can serve as a source of orthologous resistance genes. The *bc-1* on chromosome Pv03 in *P. vulgaris* and *Rsv4* on Gm02 in *G. max* are syntenic gene regions that confer resistance to *Potyviruses*. Receptor-like kinases underlie both gene regions, and a linked RNase H-Like protein, which enhances potyvirus resistance, was recently associated with *Rsv4*. We sought to determine if *RNase H-Like* encoding genes are similarly located in the *bc-1* region and enhance resistance to BCMNV. Synteny analysis between *P. vulgaris* and *G. max* revealed *RNase H-Like* genes near the *bc-1* locus. Positional cloning among reference genomes and select genotypes, along with linkage mapping in recombinant inbred line and F_2 populations, identified the *RNase H-Like 1* gene, which enhanced resistance to BCMNV NL-3 strain when combined with *I*, *bc-1*, and *bc-u* or with *bc-1* and *bc-u* genes by reducing systemic spread of susceptible symptoms in non-inoculated leaves. A single nucleotide polymorphism marker, G03_4166082, was developed to track the resistant and susceptible alleles for *RNase H-Like 1* in breeding programs. Overall, this study advances the understanding of the complex mechanisms underlying BCMNV resistance in common bean.

Plain Language Summary

Previous independent genomic and molecular studies revealed an *RNase H-Like* and a malectin receptor-like kinase (*GmMLRK1*) as candidate genes for the *Rsv4* locus conferring resistance to *soybean mosaic virus* in soybean. Previous studies in dry bean revealed a receptor-like kinase as a candidate gene for *bc-1* locus conferring resistance to *bean common mosaic virus* and *bean common mosaic necrosis virus*

Abbreviations: ADP, Andean diversity panel; BCMNV, *Bean common mosaic necrosis virus*; BCMV, *Bean common mosaic virus*; DDP, Durango diversity panel; dTN, delayed top necrosis; HG, host group; mM, mild mosaic; PG, pathogroup; RIL, recombinant inbred line; RLK, receptor-like kinase; SMV, *Soybean mosaic virus*; SNP, single nucleotide polymorphism; TN, top necrosis; VN, vein necrosis.

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(BCMNV), which is syntenic to *Rsv4* gene region in soybean. In this study, we identified three uncharacterized *RNase H-Like 1–3* genes near the *bc-1* by using the sequence of an RNase H family member within the *Rsv4* region in soybean as a search tool. One of the genes, *RNase H-Like 1*, had a mutation, which co-segregated with enhanced resistance to BCMNV. We developed a marker for the mutation that breeders can use to develop cultivars with improved resistance to BCMNV.

1 | INTRODUCTION

Bean common mosaic virus (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) are related positive-sense RNA *Potyviruses* that infect common bean (*Phaseolus vulgaris* L.) globally. These viruses are spread by various aphid species nonpersistently and through infected seeds. Yield loss in common bean fields caused by BCMV and BCMNV can exceed 80% (Morales, 2003). The primary strategy for controlling these viruses is through host plant resistance. Drijfhout (1978) initially described a resistance model involving six recessive resistance alleles (*bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, *bc-3*, and *bc-u*) and the dominant *I* gene in 11 host groups (HGs) with isolate diversity categorized into seven pathogroups (PG). Later studies added PG-VIII with strain 1755a that overcomes *bc-2* and *bc-3* but not the *bc-1* resistance gene (Feng et al., 2015), and HG-12 with genotypes possessing both dominant *I* and recessive *bc-3* genes (Larsen et al., 2005).

Soler-Garzón et al. (2021a, 2021b, 2023) updated the recessive gene model (*bc-1*, *bc-2*, *bc-3*, *bc-u*^{d}, and *bc-u*^{r}), revealing that the *Bc-1* and *Bc-2* loci each harbor a single resistance allele (*bc-1* and *bc-2*). Additionally, the *bc-u* helper gene exhibited two alleles (*bc-u*^{d} and *bc-u*^{r}) with distinct cross-over reactions against different BCMV/BCMNV PGs in combination with other resistance genes. The *bc-u* and *bc-2* loci are attributable to homologous genes on *P. vulgaris* chromosomes Pv05 and Pv11, respectively, encoding vacuolar protein-sorting 4 (*Vps*4) AAA+ ATPase proteins, essential components of the endosomal sorting complexes required for transport (Soler-Garzón et al., 2021b, 2023). The *bc-3* gene on chromosome Pv06 encodes an eIF4E translation initiation factor, which plays a crucial role in supporting viral RNA translation and, consequently, replication (Hart & Griffiths, 2013; Naderpour et al., 2010).

For *bc-1*, two receptor-like kinases (RLKs) on chromosome Pv03 were identified as candidate genes (Soler-Garzón et al., 2021a). The genomic region for *bc-1*, including both RLKs, exhibited synteny with a region on chromosome Gm02 in soybean associated with the *Rsv4* gene (Hayes et al., 2000; Ilut et al., 2016; Maroof et al., 2010) that conditions resistance to *Soybean mosaic virus* (SMV), also a potyvirus.

Recently, Che et al. (2023) identified a malectin-like receptor kinase *GmMLRK1* as a candidate gene for *Rsv4* in soybean that was overexpressed in response to SMV infection, resulting in reduced viral accumulation and localized hypersensitive response. Conversely, *GmMLRK1* knockout mutants were susceptible to SMV. In addition to *GmMLRK1*, an *RNase H-Like* protein that degrades viral double-stranded RNA (dsRNA) was identified as a candidate gene for the *Rsv4* locus (Ishibashi et al., 2019) using positional cloning, transient expression in susceptible soybean, and mutagenesis in resistant soybean. In soybean, the genes encoding *GmMLRK1* and *RNase H-Like* are tightly linked (~5 kb). However, they function independently, with *GmMLRK1* exhibiting a major effect, which is slightly enhanced in the presence of *RNase H-Like* (Che et al., 2023).

Based on the resistance effect of a gene encoding an *RNase H-Like* protein in *Glycine max* against SMV, we sought to examine genes encoding orthologous *RNase H-Like* proteins in the *bc-1* genomic region in *P. vulgaris* for resistance effect against BCMNV infection.

2 | MATERIALS AND METHODS

2.1 | BCMNV reaction

Common bean genotypes phenotyped for BCMNV reaction in this study included a recombinant inbred line (RIL) population of 142 RILs, derived from a cross between Rojo (HG-10) and CAL 143 (HG-3), from a previous study (Tock et al., 2017). Additionally, 19 accessions from the Durango diversity panel (DDP) (Soler-Garzón et al., 2021a) and 7 accessions from the Andean diversity panel (ADP) (Cichy et al., 2015) were screened. Furthermore, 74 F_2 individuals from reciprocal crosses between Amanda (HG-10) and Blush (HG-9), along with HG cultivars Top Crop (HG-9) and Beryl (HG-10), and reference genomes G19833 (HG-2), GN 1140 (HG-3), and UI 111 (HG-4) were included.

Genotypes were inoculated with BCMNV strain NL-3 D (henceforth NL-3) from PG-VI, a prominent isolate used by breeders (Kelly, 1997), because it differentiates lines with gene combinations exhibiting mosaic (as in HGs, HG-1, -2, -4, and -5), mild mosaic (HG-3), top necrosis (HG-8 and -9), restricted vein necrosis (HG-10), necrotic local lesions (HG-11), or no symptoms (HG-6, -7, and -12). Inoculations

were conducted under controlled greenhouse conditions at the USDA-ARS facility in Prosser, WA, with temperatures ranging from 22˚C to 28˚C and 14-h photoperiod, using artificial lights as necessary. Each genotype was grown in 9-cm^3 pots, with three seeds per pot (only one seed for the F_2 population), using a commercial potting mix (Sun Gro Horticulture). Approximately 10 days after planting, when the primary leaves were 80%–100% fully expanded, the plants were mechanically inoculated with NL-3 strain, according to the method used by Drijfhout (1978). Phenotypic data were recorded weekly from 1 to 4 wpi (weeks postinoculation) based on visual characterization and differential cultivar reactions. Plant reactions to NL-3 strain inoculations were categorized based on HG response as follows (Figure S1):

M, severe systemic mosaic mottle symptoms, accompanied by leaf deformation and plant stunting in HG-2; *mM*, mild systemic mosaic mottle symptoms observed in HG-3; *TN*, lethal systemic top necrosis by 7–10 dpi (days postinoculation), leading to plant death in HG-9; and *VN*, vein necrosis restricted to the inoculated leaves in HG-10.

Additionally, the following symptoms were observed in RILs and F_2 plants in this study:

mM$^+$, mild mosaic on inoculated leaves, accompanied by a yellow pattern with green patches on non-inoculated leaves, forming a mosaic-like appearance between 2 and 4 wpi; *VN*$^+$, restricted vein necrosis on inoculated leaves, with some small patches (10 mm^2) of systemic restricted vein necrosis appearing on upper trifoliolate leaves between 2 and 4 wpi; and *dTN*, delayed top necrosis, beginning 11 dpi, often leading to plant death.

In response to NL-3, necrotic symptoms reveal level of resistance VN > VN$^+$ > dTN > TN in the presence of *I* gene as do mosaic symptoms mM > mM$^+$ > M in the absence of *I* gene.

2.2 | Synteny analysis of *RNase H-Like* genes in *bc-1* region

Gene sequences NM_001249088 (NCBI accession number) and NM_001253944, which encode RNase H-Like proteins (referred to as *RNase HLike* genes henceforth), were identified by Ishibashi et al. (2019) in *G. max* (reference genome Williams 82; Wm82.a6.v1) near the *Rsv4* locus on chromosome Gm02, which is orthologous to the *bc-1* locus on Pv03 in *P. vulgaris* according to Soler-Garzón et al. (2021a) and which fits the general synteny observed between *P. vulgaris* and *G. max* chromosomes by McClean et al. (2010).

In this study, the NM_001249088 and NM_001253944 sequences were used as queries to identify genes encoding *RNase H-Like* proteins in *P. vulgaris*. The analysis was conducted using the *P. vulgaris* reference genomes G19833 *v*2.1 (Schmutz et al., 2014), which carries *bc-1* and *Bc-u* (wild type) alleles, and UI 111 *v*.1.0, which carries *bc-2*$^{[UI111]}$ and *bc-u*^{r} alleles. These genomes were downloaded from the Phytozome *v*13 database. Additionally, the whole-genome sequence of the GN 1140 genotype, which carries *bc-1* and *bc-u*^{d} alleles, was sequenced and assembled by McClean et al. (2022) and included in this analysis.

Synteny analysis between *bc-1* and *Rsv4* regions was performed, and a plot was generated using GeneBank (gbk) format files as input for pyGenomeViz *v*0.2.1 (mode: pgv-mummer) with an identity threshold of 50% (https://github.com/moshi4/pyGenomeViz). *RNase H-Like* gene prediction was initially performed for the *bc-1* region using AUGUSTUS *v*3.1 software (Stanke et al., 2006). To improve the accuracy of gene model annotations and identify unannotated coding regions, RNA-seq and the Log-Scale RNA-Seq data from Phytozome *v*13 database were integrated, providing additional mapping information specific to *P. vulgaris*.

Core Ideas

- *bc-1* and *Rsv4* are syntenic gene regions in *Phaseolus vulgaris* and *Glycine max* that confer resistance to *Potyviruses*.
- Similar to *Rsv4*, the resistance conferred by *bc-1* is enhanced by an *RNase H-Like* gene.
- *RNase H-Like 1* gene reduces systemic spread of the virus in dry bean genotypes with *bc-1* and other resistance genes.

2.3 | Toward identification of *RNase H-Like* candidate gene

The Andean common bean parents Rojo and CAL 143 of the "RC" RIL population are fixed for *bc-1* and *bc-u*^{d} resistance genes, with only Rojo carrying the *I* gene. For the RILs that carried the *I* gene, slight differences were observed in phenotypic response to BCMNV NL-3 strain. Some lines exhibited VN and others exhibited VN$^+$ response. We hypothesized that the difference in response may be caused by an *RNase H-Like* gene. Therefore, we searched for non-synonymous polymorphic mutations between the RC RIL parents for any *RNase H-Like* genes found in the *bc-1* region.

Genomic DNA from Rojo and CAL 143 was extracted using the Qiagen DNeasy Plant Pro kit, yielding DNA fragments of approximately 8 kb. Additionally, genomic DNA from the ADP and DDP accessions used in this study for single nucleotide polymorphism (SNP) marker analysis was

extracted from 20 mg of leaf tissue from individual plants and isolated using the Qiagen DNeasy 96 Plant Kit.

DNA sequencing for Rojo and CAL 143 was performed on an R10.4.1 flow cell (FLO-MIN114) using the SQK-NBD114.24 kit, with a sequencing duration of 72 h. Pod5 files were processed using Guppy GPU *v*6.5.7 (dna_r10.4.1_e8.2_400 bps_5khz_sup.cfg) on the Kamiak cluster at Washington State University to trim adapters and converted to FASTQ format (Benton, 2021). FASTQ files were cleaned using Nanofilt software (De Coster et al., 2018) with the parameters -q 9 –headcrop 5 –tailcrop 5 -l 500 and mapped to the G19833 *v*2.1 reference genome using Minimap2 *v*2.26 (option: -ax map-ont) (Li, 2018).

BAM files were generated using Samtools *v*1.15.1 (Li et al., 2009) and sorted with Picard tools *v*2.21.4 (Broad Institute, 2019; https://broadinstitute.github.io/picard/). Subsequently, BAM files were polished using PEPPER_deepvariant (Shafin et al., 2021), and a new index (.bai) file was generated using samtools *index* command. The *bc-1* region sequences were extracted with the Samtools *consensus* command (parameters: –show-ins yes, –min-depth 2, –cutoff 20, and –mode Bayesian) and aligned to G19833 *v*2.1 and GN 1140 sequences using Geneious software *v*9.1.8 (Kearse et al., 2012) for variant calling.

2.4 | Mutation-specific marker design

Non-synonymous polymorphic variants between Rojo and CAL 143 parents were identified within any *RNase H-Like* gene near *bc-1* region. Among the variants, one SNP marker was developed to track the *RNase H-Like 1* gene across the 142 RC RILs, and the full list of genotypes is described above. The RC RIL genetic linkage map, built using 1630 SNP markers aligned with the *P. vulgaris* G19833 *v*2.1 reference genome (Miklas et al., 2020; Tock et al., 2017), was used to examine co-segregation of the SNP marker within the *RNase H-Like 1* gene with phenotypic response to BCMNV. Residual DNA of the 142 RILs from previous studies was used for marker genotyping.

The RILs and parents were also evaluated for presence of resistance-linked markers developed by Soler-Garzón et al. (2021a, 2023) for *I* (S02_48908259), *bc-1* (S03_4203361), and *bc-u*^{d} (IND_05_36225873) genes. SNP marker genotyping was performed using the Tm-shift protocol (Wang et al., 2005), with amplification by PCR on an Eppendorf Mastercycler (Eppendorf). The PCR reaction volume was 20 µL, consisting of 1× Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.1 µL Taq polymerase (Promega), 0.15 µM of each primer (synthesized by IDT), 1× EvaGreen dye (Biotium), and 20 ng extracted genomic DNA. The thermal cycling profile included an initial denaturation at 94°C for 2 min, followed by 38 cycles of denaturation at 92°C for 20 s, annealing for 20 s (i.e., annealing temperature was optimized for each primer pair), and extension at 72°C for 20 s. A final extension was conducted at 72°C for 5 min.

For allele determination, DNA melting analysis was performed using a QuantStudio 5 real-time PCR system (Applied Biosystems) with EvaGreen fluorescence detection. The melt curve data were collected at a ramp rate of 0.04°C/s from 70°C to 95°C.

2.5 | Allelism test for *RNase H-Like 1* gene

F_2 reciprocal populations were developed by crossing the snap bean cultivar Amanda and the Andean light red kidney bean Blush. Both cultivars have fixed *I* and *bc-1* genes. However, Amanda carries a *bc-u* gene, exhibiting vein necrosis symptoms (HG-10), while Blush (similar to HG-9 cultivar Top Crop) without *bc-u* exhibits top necrosis reaction to BCMNV NL-3 strain.

DNA from each F_2 plant was extracted from four-leaf disks (approximately 30 mm^2) using an alkaline-based extraction method adapted from Xin et al. (2003). In this protocol, Buffer A (50 mM NaOH and 1% Tween 20) was used to disrupt the cell walls, while Buffer B (100 mM Tris-HCl and 1.7 mM EDTA at pH 8) facilitated DNA neutralization and protein precipitation. The extracted DNA was diluted at a 1:5 ratio, consisting of 1 µL of DNA and 5 µL of nuclease-free water. Then, 5 µL of this DNA dilution was used as a template for PCR.

3 | RESULTS

3.1 | Synteny analysis of *RNase H-Like* genes linked to *bc-1*

Sequence comparison and gene prediction, across the three *P. vulgaris* and one *G. max* reference genome, identified three *RNase H-Like* genes (named 1–3) in the *bc-1* region of *P. vulgaris* G19833 and GN 1140. Additionally, one *RNase H-Like* gene was found in *P. vulgaris* UI 111, and at the syntenic *Rsv4* locus in *G. max* Wm82, two *RNase H-Like* genes were identified (Figure 1). Among *P. vulgaris* reference genome genotypes, G19833 was susceptible to NL-3 strain with mosaic symptoms (M) and possesses *bc-1* gene and *Bc-u* wild type allele, GN 1140 exhibited mild systemic mosaic mottle symptoms (mM) possessing *bc-1* + *bc-u*^{d}, and UI 111 showed mosaic symptoms (M) against NL-3 strain and possessed *bc-u*^{r} + *bc-2*$^{[UI\ 111]}$.

The *RNase H-Like* genes were identified within an intergenomic region, flanked by three annotated gene models across reference genomes linked to *bc-1* and *Rsv4* loci. The

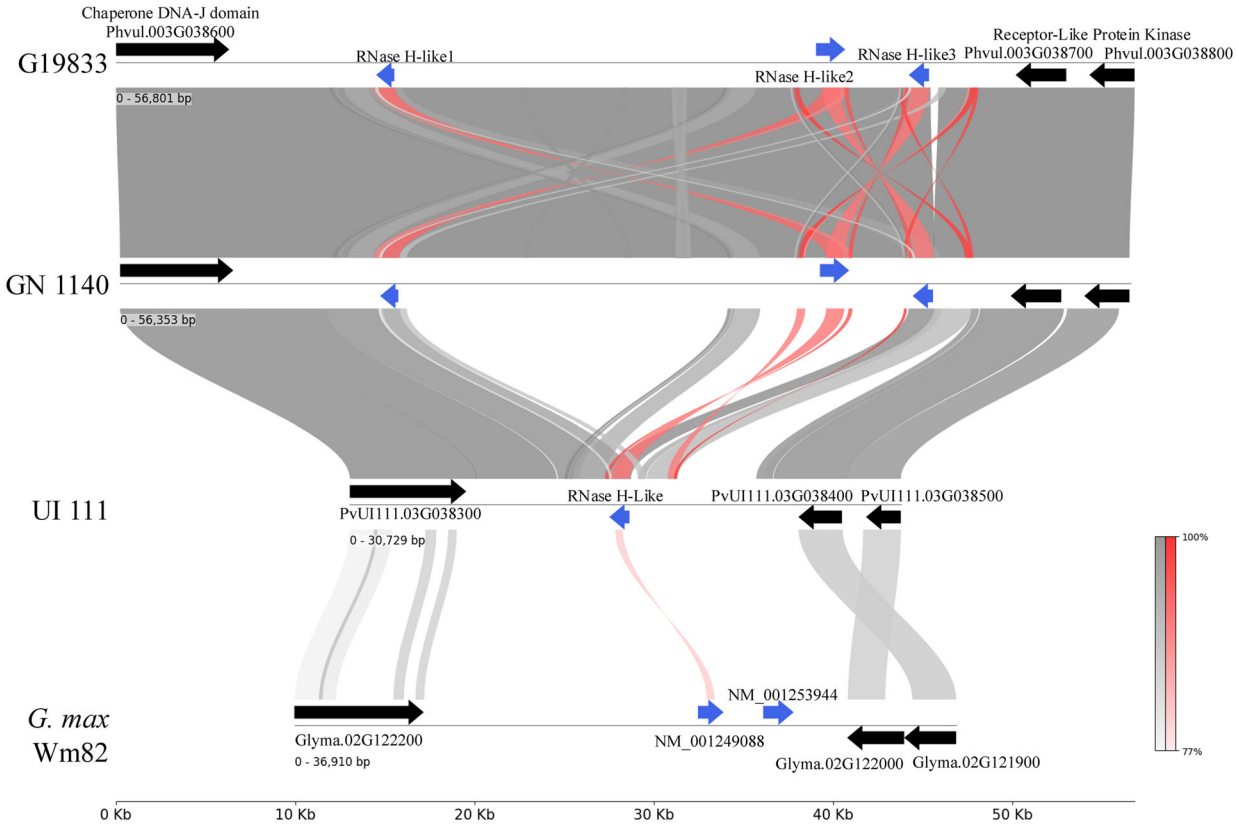

FIGURE 1 Comparison reference genomes for *bc-1* (*Phaseolus vulgaris*) and *Rsv4* (*Glycine max*) regions associated with resistance to *Bean common mosaic necrosis virus*/*Bean common mosaic virus* (BCMNV/BCMV) and *Soybean mosaic virus* (SMV), respectively. Gene models (black arrows) and unannotated *RNase H-Like 1–3* genes (blue arrows) with assembled spliced alignments obtained from Phytozome *v*13.

flanking gene models in G19833 *v*2.1 and UI 111 reference genomes encoded a chaperone DNAJ-domain superfamily protein (Phvul.003G038600/PvUI111.03G038300) and two RLK proteins (Phvul.003G038700/PvUI111.03G038400 and Phvul.003G038800/PvUI111.03G038500) on chromosome Pv03 (Table 1). These genes were found to be orthologous to the *G. max* gene models Glyma.02g122200, Glyma.02G121900, and Glyma.02G122000, located on chromosome Gm02.

Computational analysis of the in silico translated *RNase H-Like* proteins, derived from open reading frames predicted by AUGUSTUS software and compared to available RNA-seq data from the Phytozome database, revealed *RNase H-Like 1* with 99% identity between G19833 and GN 1140, 100% identity for *RNase H-Like 2* between G19833 and GN 1140, and *RNase H-Like 3* showed 100% identity between G19833 and GN 1140 and 95% identity with the unique copy of the *RNase H-Like* gene in UI 111 (Figure 2A). These *RNase H-Like* genes in *P. vulgaris* exhibited highest similarity to the NM_001249088 *RNase H-Like* gene from *G. max* Wm82.a6.*v*1 reference genome, with protein identities ranging from 53% to 62%.

Notably, *RNase H-Like 1* gene, located in a negative-sense orientation, spanned two exons between 4,165,791 and 4,166,613 bases on Pv03 in G19833 *v*2.1 reference genome. Seven nonsynonymous mutations were identified in the second exon between G19833 and GN 1140. Furthermore, nanopore sequencing of Rojo and CAL 143 to 4× coverage revealed that Rojo carried the *RNase H-Like 1* GN 1140 haplotype, while CAL 143 displayed the G19833 haplotype (Table 2). The translated *RNase H-Like 1* protein contained 245 amino acids (aa) and possessed five amino acid changes located within the *RNase H-Like* domain from 92 to 215 aa (Figure 2B).

3.2 | Mutation-specific marker design for *RNase H-Like 1* gene

A robust trio of primers was designed for amplification of variants in *RNase H-Like 1* gene, based on the alignment of *RNase H-Like 1*, *RNase H-Like 2*, and *RNase H-Like 3* genomic sequences from G19833 and GN 1140 genotypes, minimizing the inclusion of repetitive regions. The nonsynonymous SNP mutation located at Pv03: 4,166,082 bases (G19833 *v*2.1 reference genome) within *RNase H-Like 1* was specifically targeted using the following primer set: Forward A (gcgggcGCTGCAGCTTGTTTCAA), Forward B (gcgggcagggcggcGCTGCAGCTTGTTTCAG), and a common reverse (TCATGGTGATTGGGCTTAAC) (Table

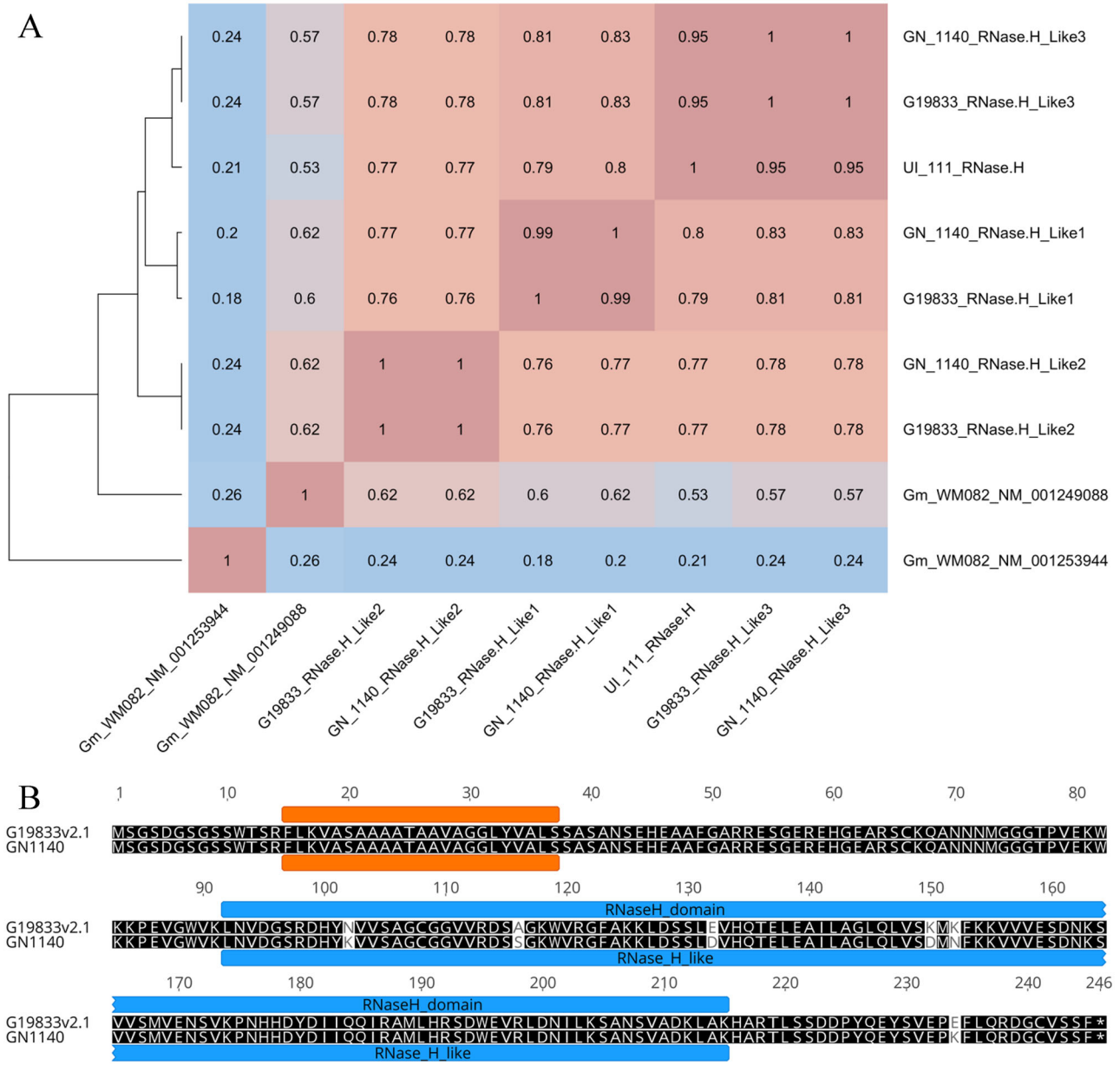

FIGURE 2 Phylogenetic analysis and protein characterization of *RNase H-Like* proteins across *Phaseolus vulgaris* and *Glycine max* reference genomes. (A) Phylogenetic relationship and identity matrix values of *RNase H-Like* proteins between *P. vulgaris* G19833 *v*2.1, GN 1140, and UI 111 *v*1.0, and *G. max* Wm82.a6.*v*1 reference genomes. (B) *RNase H-Like 1* protein showing amino acid changes between G19833 *v*2.1 and GN 1140, both possessing *bc-1*. Predicted membrane-bound protein region is indicated by an orange bar, while the *RNase H-Like* domain is represented by a blue bar. Identical amino acids are colored black, while uncolored amino acids are not similar.

S1). This SNP marker, named G03_4166082, is a putative gene/mutation-specific marker for *RNase H-Like 1* and is ~37 kb upstream from the S03_4203361 SNP marker linked to a *bc-1* RLK candidate gene described by Soler-Garzón et al. (2021a). Moreover, two SNPs were identified within the 105-base amplicon of the G03_4166082 marker, located at Pv03: 4,166,074 and Pv03: 4,166,080 bases. Lines genotyped with G03_4166082 and carrying the CC favorable allele, such as GN 1140 and Rojo, displayed a higher melting temperature (Tm) peak at approximately 84˚C. In contrast, lines with the TT negative allele, such as G19833 and CAL 143, showed a Tm peak around 82.7˚C. No amplification was observed in lines lacking *RNase H-Like 1* gene as in the UI 111 genotype (Figure 3).

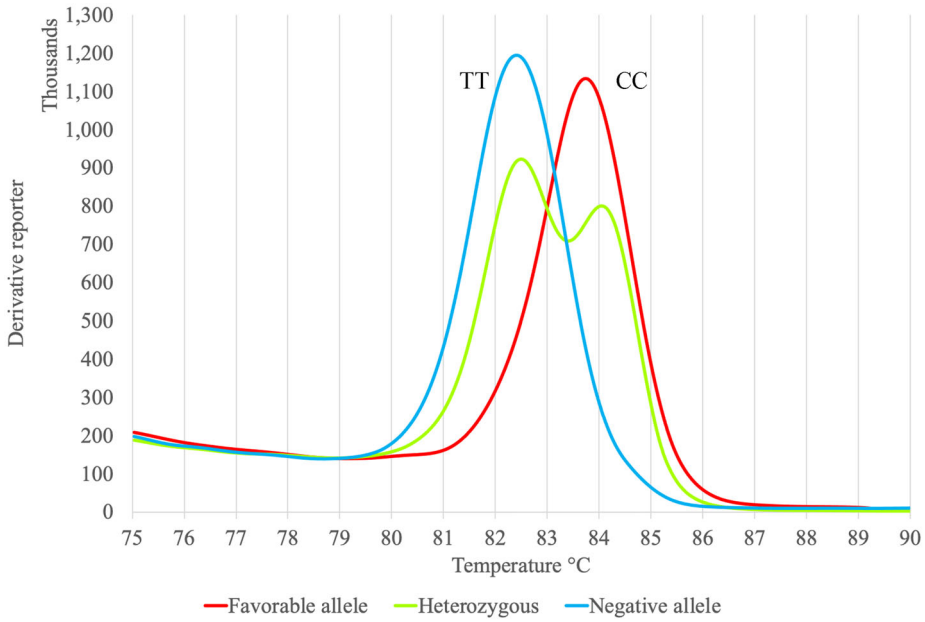

FIGURE 3 Melting curve analysis of the G03_4166082 single nucleotide polymorphism (SNP) marker, developed for detecting the *RNase H-Like 1* gene in common bean using Tm-shift primers. Melting curve for the favorable allele (CC) is shown in red, the negative allele (TT) is shown in blue, and the heterozygous displaying both alleles (CT) is shown in green.

3.3 | Genetic mapping of *RNase H-Like 1* gene

The parental lines of the RC RIL population, evaluated with *I*, *bc-1*, and *bc-u*^{d} gene markers developed by Soler-Garzón et al. (2021a, 2021b, 2023) and G03_4166082 marker for *RNase H-Like 1*, showed distinct genotypic and phenotypic traits. The parent Rojo homozygous for *I*, *bc-1*, *bc-u*^{d}, and the *RNase H-Like 1*^{C} favorable allele, denoted by superscript "C," exhibited VN against BCMNV NL-3 strain. In contrast, CAL 143, homozygous for *bc-1*, *bc-u*^{d}, and the unfavorable *RNase H-Like-1*^{T}, denoted by superscript "T," showed mM^{+} reactions. Among the RILs, in addition to the parental phenotypes, VN^{+} and mM reactions were observed (Figure 4).

In the RIL population, 89 RILs showed necrotic reactions, and 40 RILs had non-necrotic reactions, resulting in a distorted 2:1 segregation favoring the *I* gene ($x^2 = 0.29$, $df = 1$). Furthermore, the 89 RILs with *I* gene exhibited either VN (50 RILs) or VN^{+} (39 RILs) symptoms. In contrast, the 40 RILs lacking *I* gene exhibited mM (22 RILs) or mM^{+} symptoms (18 RILs). Note that 13 RILs were excluded due to segregating symptoms or poor germination.

For genetic linkage mapping purposes, RILs with VN and mM phenotypes were coded "BCMNV-R," representing the favorable allele with the letter "A" from Rojo, and RILs with VN^{+} or mM^{+} phenotypes were coded "BCMNV-S," representing the unfavorable allele with the letter "B" from CAL 143. Genetic mapping in the RC population revealed that the phenotypic marker BCMNV-R/BCMNV-S was located on chromosome Pv03 in a 470 kb interval from 3.92 to 4.39 Mb

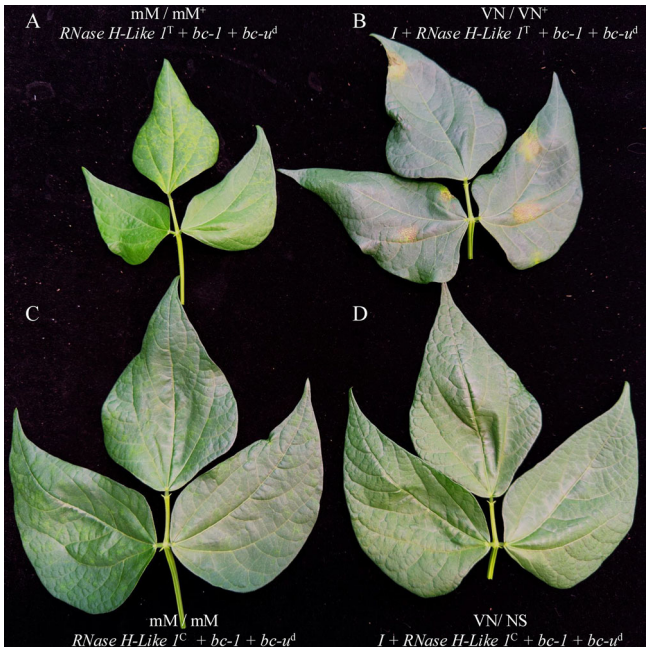

FIGURE 4 Differential *Bean common mosaic necrosis virus* (BCMNV) NL-3 reaction in non-inoculated leaves at 14–28 dpi for genotypes lacking the *I* gene (A, C) and genotypes with the *I* gene (B, D) but fixed for *bc-1* and *bc-u*^{d} that segregate for *RNase H-Like 1*. Phenotype reactions are presented as numerator = inoculated and denominator = non-inoculated leaves, separated by a foward slash (/) for each genotype. For the non-inoculated leaves—(A) mM^{+}, systemic mosaic mottle; (B) VN^{+}, some small patches of systemic restricted vein necrosis; (C) mM, milder systemic mosaic mottle; and (D) NS, no apparent systemic symptoms.

TABLE 1 Synteny analysis between common bean (G19833 *v*2.1 and UI 111 *v*1.0) and soybean (Wm82.a6.*v*1) reference genomes for gene models in the *bc-1* and *Rsv4* genomic regions, respectively.

Chromosome location (G19833 *v*2.1)	Annotation	G19833 *v*2.1 gene model	UI 111 *v*1.0 gene Model	*Glycine max* Wm82.a6.*v*1
Pv03: 4,151,102–4,157,419	Chaperone DNAJ-domain superfamily protein	Phvul.003G038600	PvUI111.03G038300	Glyma.02g122200
Pv03: 4,165,791–4,166,613	Ribonuclease H-Like superfamily	*RNase H-Like 1*	Absent	Absent
Pv03: 4,190,558–4,191,335	Ribonuclease H-Like superfamily	*RNase H-Like 2*	Absent	Absent
Pv03: 4,195,570–4,196,401	Ribonuclease H-Like superfamily	*RNase H-Like 3*	*RNase H-Like*	NM_001249088
–	Ribonuclease H-Like superfamily	Absent	Absent	NM_001253944
Pv03: 4,201,273–4,204,095	Receptor-Like protein Kinase	Phvul.003G038700	PvUI111.03G038400	Glyma.02G121900
Pv03: 4,205,380–4,207,902	Receptor-like protein kinase	Phvul.003G038800	PvUI111.03G038500	Glyma.02G122000

(Figure 5). This 470 kb interval overlapped the *bc-1* region from 4.02 to 4.23 Mb observed by Soler-Garzón et al. (2021a).

Additionally, the G03_4166082 SNP marker integrated into the RC genetic map was 100% correlated with the BCMNV-R/BCMNV-S phenotypic marker, whereby the favorable SNP marker allele CC co-segregated with VN and mM symptoms and unfavorable TT allele with VN^+ and mM^+ symptoms.

3.4 | Inheritance of the *RNase H-Like 1* gene

Reciprocal F_2 populations between Amanda and Blush were used to further examine the inheritance of *RNase H-Like 1* gene in common bean. The parent Amanda homozygous for *I*, *bc-1*, *RNase H-Like* 1^C (favorable resistant C allele), and an alternative *bc-u* mutation on *Vps4* candidate gene (Phvul.005G125100; G19833 *v*2.1 reference genome) different from *bc-u*^{d} but with similar function exhibited VN to NL-3 strain. The parent Blush homozygous for *I*, *bc-1, RNase H-Like* 1^T (susceptible T allele), and *Bc-u* exhibited TN.

The F_2 plants, inoculated with NL-3 strain, exhibited four different phenotypes: VN, VN^+, dTN, and TN (Table 3). No reciprocal effects were observed between the F_2 populations, so the F_2 individuals were pooled. The *I* and *bc-1* genes were in homozygous *I*//*I* and *bc-1*//*bc-1* state for all F_2 individuals, so just *RNase H-Like 1* and *Bc-u* loci were segregating. Nine genotypes were observed, and they fit a 1:2:1:2:4:2:1:2:1 dihybrid segregation ratio ($X^2 = 0.06$, $df = 8$) expected for two independent loci.

F_2 plants with the parental genotypes, Amanda and Blush exhibited expected VN and TN phenotypes, respectively. A VN^+ reaction was observed in F_2 individuals homozygous for susceptible *RNase H-Like* 1^T alleles and fixed for *bc-u*//*bc-u*. The VN^+ observed in heterozygous *RNase H-Like* 1^C//*RNase H-Like* 1^T individuals fixed for recessive *bc-u*//*bc-u* supports a dominant effect for the unfavorable *RNase H-Like* 1^T allele. All individuals with *Bc-u*//*Bc-u* genotypes (lacking a recessive *bc-u* allele) exhibited TN, regardless of allelic state at the *RNase H-Like 1* locus. Individuals fixed for *RNase H-Like* 1^T and heterozygous for *Bc-u*//*bc-u* also expressed TN. A more resistant dTN reaction was observed in heterozygous *Bc-u*//*bc-u* individuals with at least one *RNase H-Like* 1^C allele. These phenotypic reactions fit a 6 (TN):6 (dTN):3 (VN^+):1 (VN) segregation ratio ($X^2 = 0.39$; $df = 3$), supporting epistatic interaction between the genes.

3.5 | *RNase H-Like 1* SNP marker assay in panel accessions

Twenty-six common bean accessions fixed for the dominant *I* and recessive *bc-u*^{d} genes were assayed with the G03_4166082 SNP marker to further examine correlated

TABLE 2 Single nucleotide polymorphisms and amino acid (AA) changes detected within the exon regions for the *RNase H-Like 1* gene on *Phaseolus vulgaris* chromosome Pv03.

Genotype	NL-3 pheno-type	Genes	Genepool	Pv03	Pv03	Pv03	Pv03	Pv03	Pv03	Pv03
			Position	4,165,830	4,166,074	4,166,080	4,166,082	4,166,134	4,166,184	4,166,224
			AA change	E > K	K > N	K > D	K > D	E > D	A > S	N > K
G19833	M	*bc-1*	Andean	C	C	C	T	T	C	G
CAL 143	mM$^+$	*bc-1* + *bc-u*^{d}	Andean	C	C	C	T	T	C	G
Rojo	VN	*I* + *bc-1* + *bc-u*^{d}	Andean	T	G	G	C	A	A	T
GN 1140	mM	*bc- 1* + *bc-u*^{d}	Middle-American	T	G	G	C	A	A	T

Abbreviations: AA, amino acid; A, D, E, K, N, S, essential International Union of Pure and Applied Chemistry amino acid codes; M, leaf curling and plant stunting with severe systemic mosaic mottle symptoms; mM, mild systemic mosaic mottle symptoms; mM$^+$, mild mosaic on inoculated leaves and yellow pattern with patches of green on non-inoculated leaves, forming a mosaic-like appearance observed from 2 to 4 wpi (weeks postinoculation); VN, restricted vein necrosis on inoculated leaves, no systemic symptoms.

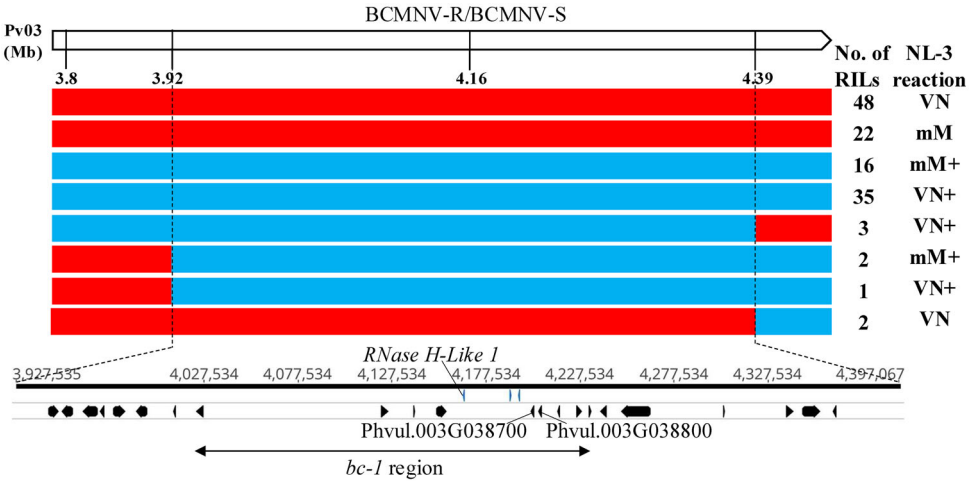

FIGURE 5 Detection and physical mapping of the *Bean common mosaic necrosis virus* (BCMNV)-R/BCMNV-S trait, associated with phenotypic response related to *RNase H-Like 1* (4.16 Mb) in the RC (Rojo/CAL 143) recombinant inbred line (RIL) population. Alleles from Rojo are indicated in red, while alleles from CAL 143 are shown in blue. The bottom track displays gene models (black arrows) identified in the G19833 *v*2.1 reference genome, overlapping the *bc-1* region identified by Soler-Garzón et al. (2021a), along with Receptor-like kinases (RLK) (Phvul.003G038700 and Phvul.003G038800) and *RNase H-Like* (blue arrows) candidate genes.

TABLE 3 Segregation of *RNase H-Like 1* (G03_4166082) and *Bc-u* linked marker alleles in combined reciprocal F_2 populations (Amanda/Blush; Blush/Amanda) with corresponding phenotypes to *Bean common mosaic necrosis virus* (BCMNV) NL-3 strain.

Cross	Segregating genes (note *I* and *bc-1* genes are fixed)	VN	VN$^+$	dTN	TN
Amanda	*RNase H-Like 1*^{C}//*RNase H-Like 1*^{C}; *bc-u*//*bc-u*	3			
Blush	*RNase H-Like 1*^{T}//*RNase H-Like 1*^{T}; *Bc-u*//*Bc-u*				3
F_2 Amanda/Blush Blush/Amanda	*RNase H-Like 1*^{C}//*RNase H-Like 1*^{C}; *Bc-u*//*Bc-u*				3
	RNase H-Like 1^C//*RNase H-Like 1*^{C}; *Bc-u*//*bc-u*			10	
	RNase H-Like 1^C//*RNase H-Like 1*^{C}; *bc-u*//*bc-u*	5			
	RNase H-Like 1^C//*RNase H-Like 1*^{T}; *Bc-u*//*Bc-u*				7
	RNase H-Like 1^C//*RNase H-Like 1*^{T}; *Bc-u*//*bc-u*			21	
	RNase H-Like 1^C//*RNase H-Like 1*^{T}; *bc-u*//*bc-u*		11		
	RNase H-Like 1^T//*RNase H-Like 1*^{T}; *Bc-u*//*Bc-u*				2
	RNase H-Like 1^T//*RNase H-Like 1*^{T}; *Bc-u*//*bc-u*				12
	RNase H-Like 1^T//*RNase H-Like 1*^{T}; *bc-u*//*bc-u*		3		

Abbreviations: dTN, delayed top necrosis beginning >11 dpi, often resulting in plant death; TN, lethal systemic top necrosis by 7–10 dpi (days postinoculation), resulting in plant death; VN, restricted vein necrosis on inoculated leaves, no systemic symptoms; VN$^+$, restricted vein necrosis on inoculated leaves, with some small patches (10 mm^2) of systemic restricted vein necrosis on upper trifoliolate leaves observed from 2 to 4 wpi (weeks postinoculation).

TABLE 4 Common bean accessions from the Andean diversity panel (ADP) and Durango diversity panel (DDP) evaluated for phenotypic reaction to *Bean common mosaic necrosis virus* (BCMNV) NL-3 strain and assayed for *RNase H-Like 1* gene-linked single nucleotide polymorphism (SNP) marker G03_4166082, and other gene-linked markers for *I*, *bc-1*, and *bc-u*[d].

ID	Accession name	Panel	NL-3 (PG-VI)	S02_48908259 Pv02: 48,908,259 *I* gene	G03_4166082 Pv03: 4,166,082 *RNase H-Like 1*	S03_4203361 Pv03: 4,203,361 *bc-1*	IND_05_36225873 Pv05: 36,225,873 *bc-u*[d]
ADP532	A 197	ADP	VN$^+$	+	−	+	+
ADP557	COS 16	ADP	VN$^+$	+	−	+	+
ADP603	Wallace 773-V98	ADP	VN	+	+	+	+
ADP604	1062-V98	ADP	VN	+	+	+	+
ADP637	Isabella	ADP	VN	+	+	+	+
ADP648	Red Kloud	ADP	VN	+	+	+	+
ADP684	Majesty	ADP	VN	+	+	+	+
DDP005	BelMiNeb-RMR-4	DDP	VN	+	+	+	+
DDP028	I06-2575-17	DDP	TN	+	Absent	−	+
DDP032	Kodiak	DDP	VN	+	+	+	+
DDP049	Baja	DDP	TN	+	Absent	−	+
DDP055	Beryl	DDP	VN	+	+	+	+
DDP056	Beryl-R	DDP	VN	+	+	+	+
DDP057	Marquis	DDP	VN	+	+	+	+
DDP068	IP08-2	DDP	VN	+	+	+	+
DDP114	USPT-CBB-1	DDP	TN	+	Absent	−	+
DDP115	USPT-CBB-3	DDP	VN$^+$	+	−	+	+
DDP116	USPT-ANT-1	DDP	TN	+	Absent	−	+
DDP133	SR9-4	DDP	VN	+	+	+	+
DDP134	GN9-4	DDP	TN	+	Absent	−	+
DDP137	Lariat	DDP	TN	+	Absent	−	+
DDP141	ND041062-1	DDP	TN	+	Absent	−	+
DDP142	ND060197	DDP	TN	+	Absent	−	+
DDP145	CDCWM-2	DDP	TN	+	Absent	−	+
DDP152	Gemini	DDP	TN	+	Absent	−	+
DDP154	UCD 9634	DDP	TN	+	Absent	−	+

Note: Plus sign (+) represents presence of the favorable resistance alleles, and minus (−) and no amplification (Absent) represent absence of the resistance alleles.
Abbreviation: PG, pathogroup; TN, lethal systemic top necrosis by 7–10 dpi (days postinoculation), resulting in plant death. VN, restricted vein necrosis on inoculated leaves, no systemic symptoms; VN$^+$, restricted vein necrosis on inoculated leaves, with some small patches (10 mm^2) of systemic restricted vein necrosis on upper trifoliolate leaves observed from 2 to 4 wpi (weeks postinoculation).

response of *RNase H-Like 1* alleles with phenotypic reaction to BCMNV NL-3 strain in different genetic backgrounds (Table 4). All 12 accessions with *RNase H-Like 1*^{C} and *bc-1* combination exhibited VN. Conversely, three accessions with *bc-1* and the unfavorable *RNase H-Like 1*^{T} allele exhibited the less resistant VN$^+$ reaction. The remaining 11 accessions lacking both *bc-1* and the favorable *RNase H-Like 1*^{C} allele because the gene was absent altogether exhibited TN. Overall, the resistance genes *RNase H-Like 1*^{C} and *bc-1* were linked primarily in coupling (cis) phase across the tested accessions with just three exceptions: A 197, COS 16, and USPT-CBB-3.

4 | DISCUSSION

Three uncharacterized *RNase H-Like 1–3* genes were identified near the *bc-1* locus in *P. vulgaris* through a BLAST search against an RNase H family member associated with the *Rsv4* locus in soybean (Ishibashi et al., 2019). The synteny between *bc-1* and *Rsv4* gene regions in *P. vulgaris* and *G. max* was previously established (Soler-Garzón et al., 2021a), and it is further strengthened by the presence of orthologous *RNase H-Like* genes within the same region in both species. The broad-spectrum resistance against similar *Potyviruses* conferred by both gene regions supports functional synteny.

The *bc-1* locus conditions resistance to BCMV, BCMNV, and *Peanut mottle virus* (PeMoV) (Larsen & Miklas, 2010), while *Rsv4*, conferring resistance to SMV, also provides resistance to BCMV (Liu et al., 2021), and transient expressed resistance to PeMoV, *Potato virus Y*, and other *potyviruses* (Ishibashi et al., 2019).

Two independent studies identified separate candidate genes for *Rsv4* in *G. max*, an *RNase H-Like* gene with 3.6 kb deletion identified by Ishibashi et al. (2019), and then a malectin-like receptor kinase *GmMLRK1* (haplotype 1) by Che et al. (2023). However, only the latter study tracked both candidate genes, revealing *GmMLRK1* had a major resistance effect against SMV that was enhanced slightly by the presence of *RNase H-Like* resistance gene. For *P. vulgaris*, we observed a similar reaction. A RLK (*PvRLK*) was identified as the candidate gene for *bc-1* (Soler-Garzón et al., 2021a), and in this study, we identified an *RNase H-Like 1* gene that enhanced the resistance effect of *bc-1*.

Although the *PvRLK* and *RNase H-Like 1* (this study) candidate genes in the *bc-1* region were solely identified by fine mapping and positional cloning of natural variants, they are supported by positional cloning, gene transcription, transformation, and knockout mutant studies conducted in soybean that identify similar candidate genes for *Rsv4* region.

RLK proteins play a vital role in the interaction networks between plants and viruses (Macho & Lozano-Duran, 2019; Tang et al., 2017). These proteins interact with viral movement proteins, promoting viral movement through tubules within plasmodesmata (Amari et al., 2010). In contrast, RNase H-Like proteins selectively degrade dsRNA in a manganese-dependent manner by targeting viral dsRNA formed during replication (Ishibashi et al., 2019). Overall, the *bc-1* locus has been shown to influence the systemic spread of BCMV in common bean (Feng et al., 2017, 2018).

For soybean, reaction to SMV was measured quantitatively (Che et al., 2023), whereas for common bean, levels of resistance were measured by distinct phenotypes VN and mM versus VN^+ and mM^+, respectively. Detection of lines for specific *RNase H-Like 1* alleles with the NL-3 strain of BCMNV required the presence of other resistance genes. When *I* + *bc-1* + *bc-u*^{d} (or a similar *bc-u* allele from Amanda) were present, the *RNase H-Like 1*^{C} favorable allele prevented visible systemic viral movement of NL-3 to non-inoculated leaves. In line with *bc-1* + *bc-u*^{d}, the mild mosaic symptoms in upper leaves were less pronounced, suggesting *RNase H-Like 1*^{C} allele reduced systemic spread of the virus. For the many bean breeding programs that use NL-3 strain to select resistant materials, it is recommended that inoculation tests be extended to at least 4 wpi for plants exhibiting initial VN and mM symptoms to ensure presence of *RNase H-Like 1*^{C}. Marker-assisted selection for the SNP marker can also be used effectively to select *RNase H-Like 1*^{C}.

The present study advances the understanding of the complex mechanisms underlying BCMNV resistance in common bean. We demonstrate the complementarity of *RNase H-Like 1*^{C} with *I*, *bc-1*, and *bc-u* genes and propose a new SNP marker G03_4166082 to facilitate the introgression and tracking of *RNase H-Like 1*^{C} gene in breeding lines for enhanced resistance to BCMNV. Further research on the potential complementary effect of *RNase H-Like 1*^{C} with other resistance genes (e.g., *bc-2* and *bc-3*) and against different BCMV and BCMNV strains representing different PGs is warranted.

AUTHOR CONTRIBUTIONS

Alvaro Soler: Conceptualization; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Phillip Miklas**: Conceptualization; formal analysis; investigation; methodology; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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