Identification and characterization of candidate R gene controlling resistance to Mungbean yellow mosaic virus disease in mungbean (Vigna radiata (L.) Wilczek).

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Abstract

Mungbean (*Vigna radiata (L.)* Wilczek) is a vital legume crop in Asia, but its productivity is severely hampered by *Mungbean yellow mosaic virus* (MYMV), often leading to yield losses up to 85%. Developing MYMV-resistant cultivars through genetic intervention is a sustainable approach to disease management. In this study, 16 mungbean genotypes, including resistant and susceptible lines, were evaluated for MYMV resistance using both natural and artificial inoculation methods. Genomic DNA was isolated and amplified using two pairs of degenerate Resistance Gene Analog (RGA) primers, CYR1 and VMR1, targeting conserved NBS-LRR domains associated with disease resistance. PCR amplification confirmed the presence of MYMV resistance genes in several genotypes, notably MYB-6, MYB-7, MYB-8, MYB-9, MYB-12, DR-12, DR-3, and KKM-3. Sequencing and BLASTn analysis of the RGA amplicons revealed high similarity with known resistance genes in Vigna species. Phylogenetic analysis further validated the relationship of these candidate R genes with established MYMV resistance genes. The study demonstrates the utility of RGA-based markers for rapid identification and characterization of MYMV resistance in mungbean, providing valuable tools for marker-assisted selection and breeding of resistant cultivars.

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is an ancient and economically significant leguminous crop widely cultivated across Asia. Characterized by its rapid growth and adaptation to warm seasons, mungbean is a diploid species (2n = 22) that predominantly self-pollinates via cleistogamous flowers. The crop is believed to have originated in India (Vavilov 1926), with *Vigna radiata* var. *sublobata* Verdc. identified as its closest wild relative and progenitor (Mehandi et al. 2019).

Despite its agronomic value, mungbean productivity in India is constrained by various biotic stresses (Pratap et al. 2019). Among these, *Mungbean yellow mosaic virus* (MYMV) disease is particularly destructive, with yield losses reported up to 85% depending on infection severity and crop stage (Karthikeyan et al. 2014; Kang et al. 2005). MYMV was first reported by Nariani (1960) at the Indian Agricultural Research Institute, New Delhi, and is now recognized as a major limiting factor for mungbean cultivation in South and Southeast Asia.

MYMV is a member of the family Geminiviridae, subgroup II, genus *Begomovirus* and possesses a bipartite single-stranded DNA genome (DNA-A and DNA-B), each approximately 2.7 kb in size. These components share a conserved common region (CR) of 200–250 bp, which is nearly identical within a virus species but variable among species. While DNA-A encodes essential functions for replication and encapsidation (Rogers et al. 1986; Sunter et al. 1987), both DNA-A and DNA-B are required for infectivity (Hamilton et al. 1983). The virus is transmitted in a persistent manner by the whitefly (*Bemicia tabaci* Genn.) with the B biotype being the most prevalent vector in Asia (Prasanna et al. 2015).

Typical symptoms of MYMV infection include yellow spots or flecks, irregular yellow and green patches, reduced flowering and pods with immature, shrivelled seeds. Severe infections can result in necrosis,

shortened internodes, and stunted plants, with yield losses ranging from 10–100% (Akhtar et al. 2009).

The development of MYMV-resistant cultivars through genetic improvement is widely regarded as the most sustainable and eco-friendly approach to disease management. Plant resistance is mediated by resistance (R) genes, which encode proteins that recognize pathogen effectors and activate defence signalling (Morel and Dangl 1997). Notably, the nucleotide-binding site-leucine-rich repeat (NBS-LRR) class of R genes is the most prevalent and well-characterized in crop species (Bent et al. 1996). These genes contain conserved motifs, such as the p-loop, kinase-2, kinase-3a, and GLPL, which are critical for ATP and GTP binding (Meyers et al. 1999; Meyers et al. 2003) and can be used as molecular probes to identify homologous resistance genes across species (Singh et al. 1988).

The use of Resistance Gene Analog (RGA)-based markers has proven effective in enhancing the pace and precision of breeding for disease resistance. RGAs have been isolated and characterized in several legume species, including mungbean (Maiti et al. 2011), urdbean (Basak et al. 2005; Maiti et al. 2011), soybean (Kanazin et al. 1996; He et al. 2003), common bean (Mutlu et al. 2006), chickpea (Huettel et al. 2002; Palomiro et al. 2006), pea (Timmerman-Vaughan et al. 2000), lentil (Yaish et al. 2004), and faba bean (Palomiro et al. 2006).

Materials and Methods

Selection of Mungbean Genotypes

A total of 14 mungbean genotypes, comprising 13 MYMV-resistant and one susceptible genotype, were selected for this study. These genotypes were cultivated across three seasons- summer, rainy and winter and were also subjected to artificial inoculation in a glasshouse for one season. Resistance was assessed based on delayed symptom expression, percent disease index (PDI), and area under the disease progress curve (AUDPC) following both natural infection and artificial challenge. Five genotypes —AVMU 1698, AVMU 1699, AVMU 16100, AVMU 16101, and KPS 2 were identified as resistant to yellow mosaic disease (YMD) (Nagaraj et al. 2018). Further confirmation of resistance was performed using vector transmission by the whitefly (*Bemisia tabaci*).

In total, 16 genotypes—including the 14 original lines, two F_2-derived lines (Bengaluru lines 1 and 3), and KKM-3 (a UAS-B released variety)—were screened under natural infection at the Main Research Station (MRS), Hebbal, University of Agricultural Sciences (UAS), Bangalore, India, during 2020–2021.

Genomic DNA Isolation

Genomic DNA was extracted from 15–20-day-old seedlings using the cetyltrimethylammonium bromide (CTAB) method as described by Lodhi et al. (1994), with modifications by Maruthi et al. (2002). DNA concentration and purity were determined using a Nanodrop spectrophotometer, and samples were diluted with 1× TE buffer to a final concentration of 50 ng/µl for subsequent analyses.

Amplification of RGA-Primed Genomic Regions

Two pairs of degenerate resistance gene analog (RGA) primers—CYR1 (from mungbean) and VMR1 (from mungbean and blackgram)—were used to amplify genomic regions from the 16 mungbean genotypes (Table 1). PCR reactions were performed in a 25 µl volume containing 2 µl of each primer (forward and reverse), 0.5 µl dNTPs, 0.5 µl Taq DNA polymerase (3 units), 2.5 µl Taq buffer, 17.5 µl nuclease-free water, and 2 µl genomic DNA, using an Eppendorf thermocycler.

The PCR cycling conditions for the CYR1 primer were initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1.5 min; followed by a final extension at 72°C for 10 min. For the VMR1 primer, the conditions were: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 45 s, annealing at 48.5°C for 45 s, and extension at 72°C for 1 min; with a final extension at 72°C for 10 min.

PCR products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide in 1× TAE buffer and visualized under UV light.

Table:1 Degenerate RGA primers used to amplify genomic regions in 16 mungbean genotypes.

Identity of Marker and host	Primer sequences	Annealing temp (º C)	Reference
CYR1 (Mungbean)	F: GGGTGGNTTGGGTAAGACCAC R: NTCGCGGTGNGTGAAAAGNCT	57.0	Maiti et al., (2011)
VMYR1 (Mungbean and black gram)	F: AGTTTATAATTCGATTGCT R: ACTACGATTCAAGACGTCCT	48.5	Basak et al., (2005)

Molecular Characterization of Resistance Gene Analogs (RGAs)

PCR products corresponding to resistance gene analogs (RGAs) from mungbean samples were purified and subjected to Sanger sequencing. The resulting sequences were analysed using the NCBI BLASTn tool to determine homology with known resistance genes. For phylogenetic analysis, the obtained RGA sequences were aligned with reference resistance gene analog sequences retrieved from the GenBank database. Phylogenetic trees were constructed using the neighbor-joining method implemented in MEGA version 7.0 (Kumar et al. 2016), enabling the assessment of evolutionary relationships between the mungbean RGAs and established resistance genes from related legume species.

Result

Detection of Resistance Gene Analogs (RGAs)

PCR amplification using the CYR1 and VMR1 degenerate primers was successful confirming the presence of MYMV disease. Specifically, amplification of CYR1-primed genomic regions indicated the presence of MYMV resistance genes in genotypes MYB-6, MYB-7, MYB-8, MYB-9, MYB-12, DR-12, DR-3, and KKM-3. Similarly, VMR1-primed regions were amplified in the same set of genotypes, further confirming the presence of MYMV resistance genes. The VMR1 amplicons (~ 445 bp) were notably smaller than those generated by CYR1 (~ 1236 bp), but both markers consistently identified the same resistant genotypes (Fig. 1 and Fig. 2).

Where L = Ladder (1000bp), 1 = MYB-1, 2 = MYB-2, 3 = MYB-3, 4 = MYB-4, 5 = MYB-5, 6 = MYB-6, 7 = MYB = 7, 8 = MYB-8, 9 = MYB-9, 10 = MYB-10, 11 = MYB-11(susceptible check), 12 = MYB-12, 13 = MYB-13, 14 = KKM-3 15 = DR-1 ,16 = DR-3

Where, L = Ladder(100bp), 1 = MYB-1, 2 = MYB-2, 3 = MYB-3, 4 = MYB-4, 5 = MYB-5, 6 = MYB-6, 7 = MYB = 7, 8 = MYB-8, 9 = MYB-9, 10 = MYB-10, 11 = MYB-11 (susceptible check), 12 = MYB-12, 13 = MYB-13, 14 = KKM-3 15 = DR-1, 16 = DR-3

Molecular Characterization of RGAs

The RGA amplicons were sequenced and analyzed using NCBI BLASTn. The current RGA-CYR1 isolate had 96.71% identity with *Vigna radiata var. radiata* putative disease resistance RPP13-1ike protein 1 (XM014647468.2) and 95.41% identity with *Vigna mungo* disease resistance protein CYR1 (CYR1) mRNA, complete sequence (HQ704837.1). The current RGA-VMR1 isolate has 89.64% identity with *Vigna mungo* disease resistance protein (VMYR-1) gene, partial sequence (AY297425.2) and 94.47% identity with *Vigna mungo* disease resistance protein (YR-3) mRNA, partial sequence (EF446378.1). The isolates have 89.11% identity *Vigna radiata* viral resistance candidate (MYR-1) gene, partial sequence (AY301990.1). The phylogenetic analysis of RGA sequence was carried out together with the known RGA sequences obtained from GenBank database by using MEGA 7.0 software (Table 2 and Table 3). GenBank accession numbers of different RGA used for current isolate sequence and analysis of nucleotide sequence of RGA against yellow mosaic virus associated with mungbean showed through phylogenetic tree (Fig. 3) and (Fig. 4).

Table 2
List of RGA CYR1 used for comparison of different resistant gene sequences.

Sl. No.	RGA Analogous	Per cent identity (%)	Accession number
1	*Vigna radiata* var. radiata putative disease resistance RPP13-like protein 1	96.71	XM014647468.2
2	*Vigna mungo* disease resistance protein CYR1 (CYR1) mRNA, complete sequence	95.41	HQ704837.1
3	*Vigna mungo* disease resistance protein CYR1 (CYR1) gene, partial sequence	95.76	HQ704838.1
4	*Vigna mungo* cultivar T-9 disease resistance protein CYR1	95.61	KR350634.1
5	*Vigna mungo* cultivar WBU-108 disease resistance protein CYR1 (YR2)	97.22	EU258701.1
6	*Phaseolus vulgaris* hypothetical protein	86.53	XM007151255.1
7	*Phaseolusvulgaris*NBS-LRRdisease resistance-like protein	84.13	EU856786.1
8	*Phaseolus vulgaris* clone ContB2, complete sequence	85.10	EU931622.1
9	*Phaseolus vulgaris* NBS-LRR resistance-like protein complete sequence	84.06	AF306506.1
10	*Vigna unguiculata* putative disease resistance RPP13-like protein 1, transcript variant X2	81.81	XR003603755.1
11	*Vignaunguiculata*cultivarXiabao2 chromosome Vu04	80.54	XP098765578.1
12	*Phaseolusvulgaris*hypotheticalprotein mRNA, complete sequence	85.5	XM007143843.1

Table 3
List of RGA VMR1 used for comparison of different resistant gene sequences.

Sl. No.	RGA Analogous	Per cent identity (%)	Accession number
1	*Vigna radiata var*. radiata TMV resistance protein N-like, transcript variant X3, mRNA	96.12	XM022784106.1
2	*Vigna mungo* disease resistance protein (YR-3) mRNA, partial sequence	94.47	EF446378.1
3	*Vigna mungo* resistance protein (VMYR-2) gene, partial sequence	94.72	AY301991.1
4	*Vigna angularis* var. angularis DNA, chromosome 11, almost complete sequence, cultivar: Shumari	91.99	AP015044.1
5	*Vigna mungo* disease resistance protein (VMYR-1) gene, partial sequence	89.64	AY297425.2
6	*Vigna radiata* viral resistance candidate (MYR-1) gene, partial sequence	89.11	AY301990.1
7	*Phaseolus vulgaris* hypothetical protein mRNA, complete sequence	85.34	XM007152493.1
8	*Glycine max* strain Williams 82 clone, complete sequence	82.15	AC235351.1
9	*Vigna unguiculata* cultivar Xiabao 2 chromosome Vu03	79.02	CP039346.1
10	*Phaseolus vulgaris* hypothetical protein mRNA, complete sequence	78.66	XM007152501.1
11	*Phaseolus vulgaris* cloneputative resistance protein TIR 31 gene, partial cds	78.66	JQ313629.1
12	*Glycine max* clone, complete sequence	77.67	AC235891.2
13	*Cajanuscajan*TMVresistanceproteinN, transcript variant X4, mRNA	77.51	XM020366882.2
14	*Glycine soja* TMV resistance protein N-like, mRNA	77.43	XM028349904.1
15	*Vigna unguiculata* TMV resistance protein N-like , mRNA	77.4	XM028068349.1
16	*Abrus precatorius* TMV resistance protein N-like transcript variant X1, mRNA	76.89	XM027501909.1
17	*Vigna vexillata* disease resistance protein homolog gene, partial sequence	79.01	AF141015.1

Discussion

The identification and characterization of resistance gene analogs (RGAs) in mungbean provide critical insights into the molecular basis of resistance against Mungbean yellow mosaic virus (MYMV), a major constraint to mungbean production in Asia. The present study demonstrates that the use of degenerate primers targeting conserved domains of plant R genes—specifically CYR1 and VMR1—enables the effective detection of candidate resistance genes in diverse mungbean genotypes.

Structure and Function of Plant R Genes

Plant R genes, particularly those belonging to the nucleotide-binding site-leucine-rich repeat (NBS-LRR) class, play a pivotal role in the recognition of pathogen-derived effectors and the activation of downstream defense responses. The NBS domain is involved in ATP/GTP binding and hydrolysis, which is essential for signal transduction, while the LRR domain mediates specific protein-protein interactions, allowing plants to recognize a wide array of pathogen effectors (Sekhwal et al., 2015). The ARC (activity-regulated cytoskeletal) domain, together with the NES (nucleotide exchange site), forms the core of the nucleotide-binding region, which is crucial for the conformational changes required during defense signaling. The high degree of sequence conservation in these domains across plant species has facilitated the development of degenerate primers, such as CYR1 and VMR1, which can amplify homologous resistance gene sequences in different legumes.

Validation and Comparative Analysis of RGA Markers

In this study, the amplification of CYR1 and VMR1 markers in multiple resistant mungbean genotypes (MYB-6, MYB-7, MYB-8, MYB-9, MYB-12, DR-1, DR-3, and KKM-3) confirms their association with MYMV resistance. This is in agreement with earlier reports by Maiti et al. (2011), who demonstrated the linkage of CYR1 with MYMIV resistance in both mungbean and urdbean. Similarly, Kabi et al. (2017) and Panigrahi et al. (2016) provided evidence for the utility of CYR1 in marker-assisted selection (MAS) for yellow mosaic virus resistance. The consistency of these results across studies and species highlights the robustness and transferability of these markers.

The VMR1 marker, originally developed for blackgram (Basak et al., 2005), also proved effective in mungbean, further supporting the hypothesis that resistance gene analogs are conserved across Vigna species. However, as noted by Sowmini and Jayamani (2014), the expression of some markers may be monomorphic in certain genetic backgrounds, underscoring the importance of validating markers in target populations before deployment in breeding programs.

Implications for Breeding and Genetic Resource Management

The practical application of RGA markers in breeding programs is multifaceted. First, they enable rapid and accurate screening of breeding populations and germplasm collections for resistance alleles, significantly reducing the time and resources required for phenotypic selection. Second, the use of molecular markers circumvents the challenges posed by environmental variability and the often-

quantitative nature of disease resistance. Third, RGA markers facilitate the pyramiding of multiple resistance genes, which is essential for achieving durable and broad-spectrum resistance.

In the context of genetic resource management, the identification of diverse resistance sources and their molecular characterization enriches the genetic base available for mungbean improvement. This is particularly important given the narrow genetic diversity observed in many cultivated mungbean varieties. The conservation of RGA sequences across Vigna and other legume species also opens avenues for the introgression of resistance genes from wild relatives, leveraging the rich genetic resources present in gene banks.

Evolutionary and Functional Insights

The phylogenetic analysis conducted in this study revealed that the identified RGAs cluster closely with known resistance genes from *Vigna radiata*, *Vigna mungo*, and other legumes, indicating a shared evolutionary origin and functional conservation. Such analyses provide valuable information on the diversification and adaptation of R genes in response to pathogen pressure. The observed sequence similarity with resistance genes from other legumes, such as *Phaseolus vulgaris* and *Glycine max*, suggests that similar defence mechanisms may operate across different legume crops and that knowledge gained in one species can inform resistance breeding in others.

Furthermore, the functional validation of these RGAs, through fine-mapping, gene expression analysis, and ultimately gene cloning, will be essential to confirm their role in MYMV resistance and to elucidate the molecular mechanisms underlying plant-virus interactions. Such studies can also reveal the presence of gene clusters, gene duplications, and other evolutionary processes that shape the R gene repertoire in mungbean and related species.

Future Prospects

Looking ahead, the integration of RGA-based markers with high-throughput genotyping platforms and next-generation sequencing technologies will enable the construction of high-density genetic maps and the identification of quantitative trait loci (QTLs) associated with disease resistance. This will further enhance the efficiency of MAS and facilitate the development of mungbean cultivars with durable resistance to MYMV and other pathogens.

Additionally, the insights gained from RGA characterization can inform the design of gene editing strategies, such as CRISPR/Cas9-mediated targeted mutagenesis, to engineer novel resistance alleles or to enhance existing resistance traits.

Conclusion

The present study provides comprehensive insights into the identification and molecular characterization of candidate resistance gene analogs (RGAs) conferring resistance to *Mungbean yellow mosaic virus* (MYMV) in mungbean *(Vigna radiata)*. By employing degenerate primers (CYR1 and VMR1)

targeting conserved domains of plant resistance genes, we successfully amplified and detected RGA sequences in several MYMV-resistant mungbean genotypes. Sequence analysis and phylogenetic comparisons revealed a high degree of similarity between these RGAs and known resistance genes from *Vigna* and other legume species, underscoring the evolutionary conservation of disease resistance mechanisms within the Fabaceae.

The validation of CYR1 and VMR1 markers across diverse genotypes demonstrates their robustness and utility for marker-assisted selection (MAS) in mungbean breeding programs. These markers enable the rapid and precise identification of resistant individuals, thereby accelerating the development of MYMV-resistant cultivars. This is particularly significant given the challenges of phenotypic screening for viral diseases, which are often influenced by environmental variability and complex inheritance patterns. The deployment of RGA-based markers thus represents a powerful approach for enhancing the efficiency and accuracy of resistance breeding.

From a genetic resource perspective, the identification of novel and diverse sources of MYMV resistance enriches the available germplasm pool and broadens the genetic base for mungbean improvement. The conservation of RGA sequences across *Vigna* and related genera also highlights the potential for utilizing wild relatives and landraces as reservoirs of valuable resistance genes. This is especially relevant in the context of ongoing efforts to conserve and utilize plant genetic resources for crop improvement and adaptation to emerging biotic stresses.

Moreover, the findings of this study have important implications for understanding the molecular evolution of plant resistance genes. The clustering of mungbean RGAs with homologous sequences from other legumes suggests that common evolutionary pressures have shaped the diversification of R genes in response to pathogen challenges. Further functional validation and fine mapping of these candidate genes will provide deeper insights into the mechanisms of resistance and facilitate the cloning and transfer of effective resistance genes across species.

In conclusion, this research advances our knowledge of the genetic architecture of MYMV resistance in mungbean and provides validated molecular tools for breeding programs aimed at developing durable, virus-resistant cultivars. The integration of RGA-based marker technology with conventional breeding and genetic resource management will play a pivotal role in safeguarding mungbean productivity and sustainability. The approaches and outcomes presented here can serve as a model for resistance gene discovery and utilization in other crop species, contributing to global efforts in crop improvement and food security.

Declarations

Author Contribution

Dr. Nagaraju is my Guide during this whole research Program. Dr Ramesh and Ms. Padmaja were my members of research committee during my Master's Program. These three members helped me through

my whole research from setting up the objective to completion of manuscript. Azar has helped me in data collection and completing the technical and no technical work.

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Figures

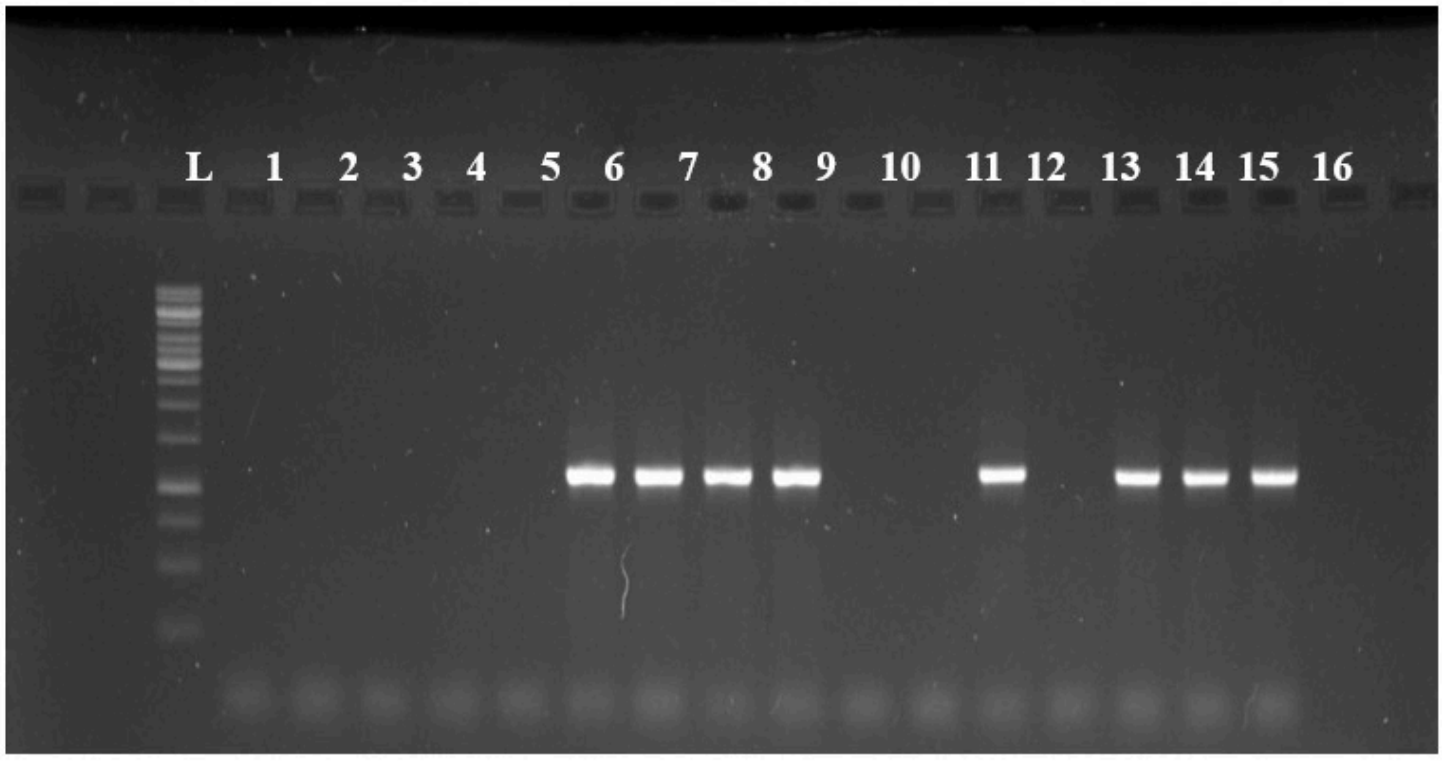

Figure 1

PCR amplification of CYR-1 marker in mungbean genotypes

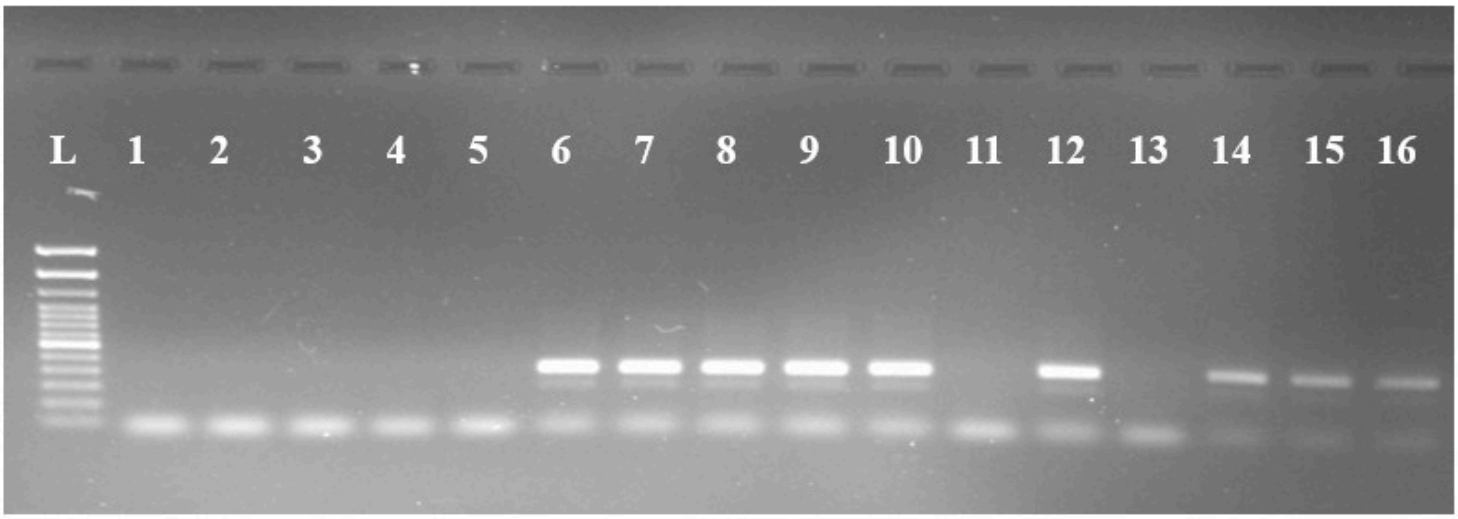

Figure 2

PCR amplification of VMR1 marker in mungbean genotypes

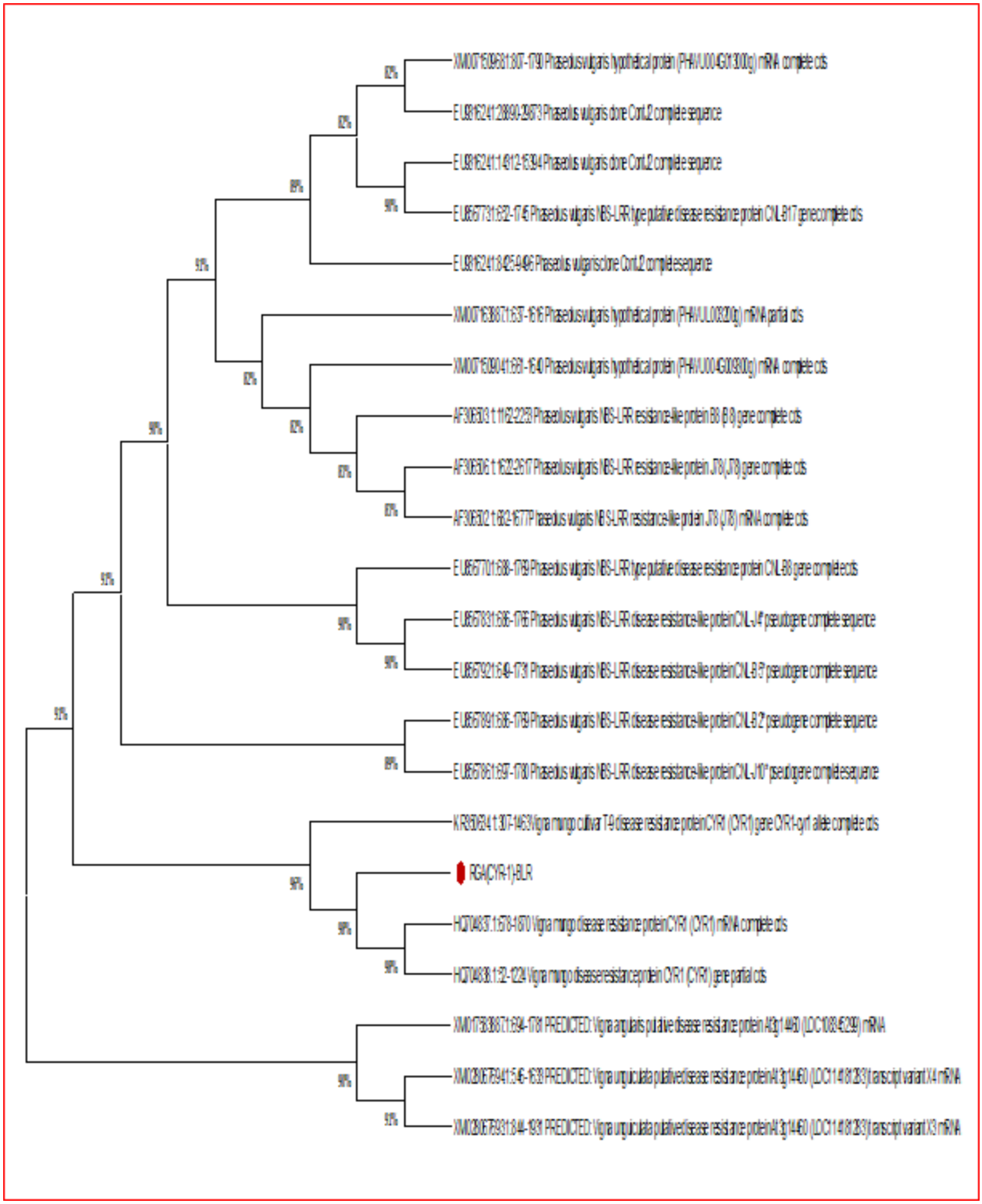

Figure 3

Phylogenetic relationship of RGA-CYR1 used for comparison of different resistant gene sequences by Neighbour joining method.

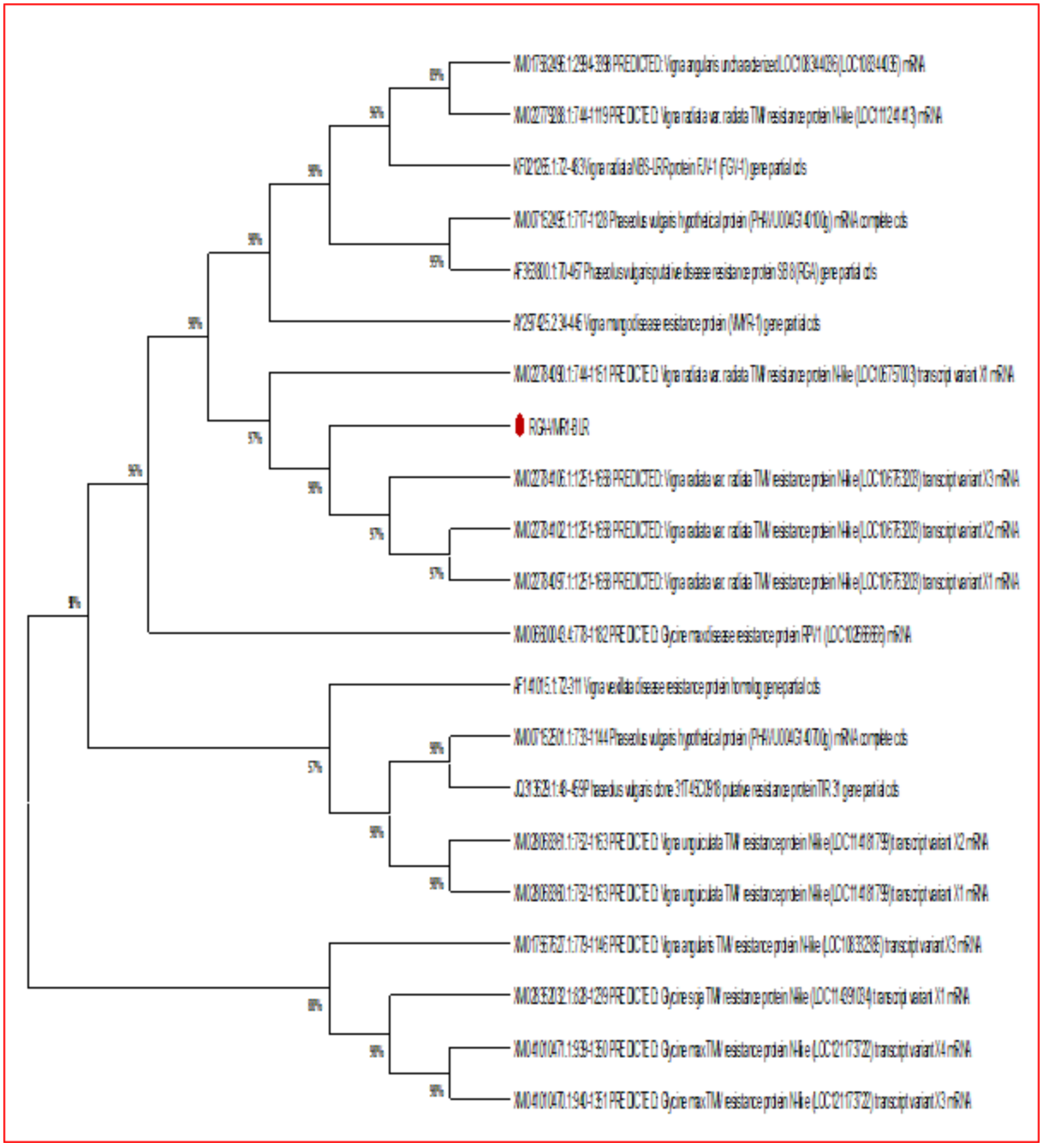

Figure 4

Phylogenetic relationship of RGA -VMR1 used for comparison of different resistant gene sequences by Neighbor joining method