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Computational genomics based probing of resistance gene analogs (RGAs) in mungbean under cercospora leaf spot disease challenge

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We investigated the genetic scenario expressed under the Cercospora leaf spot challenge in *Vigna radiata*. The resistance gene analogs (RGAs) were identified contributing to resistance against *Cercospora canescens* in the mungbean. *In silico* mining of NBS-LRR genic regions revealed 695 proteins contained NBS domain that was further characterized using bioinformatics tools to identify the regions for primer designing. The primers were screened based on genomic analysis that showed they amplified the DNA fragments in NM 92, NM 98 (resistant), and NCM251-4 (susceptible). However, in transcriptomic analysis, we did not get the amplification in NCM251-4 that showed the primers amplify disease resistance-related genes or gene sequences expressed in resistant genotypes but did not express in susceptible genotypes. The primers did not show amplification in the NCM251-4 (susceptible genotype) transcriptome, but it showed amplification in its genome, which indicates some negative regulators in susceptible genotypes that may suppress the expression of resistance genes. Three putative disease resistance gene sequences were identified under disease challenge and were named MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3 contained conserved P-loop and Kinase-2 domain and showed a 100% homology with disease resistance protein of *Vigna radiata* var radiata. Physiochemical properties of translated proteins of these RGAs showed thermal stability and hydrophilic nature. Moreover, these RGAs were predicted to belong to the P-loop and ribonuclease inhibitor superfamilies that significantly impact plants' disease resistance.
Keywords: Transcriptomics, RGAs, conserved motifs, mungbean, disease resistance.

INTRODUCTION

In natural habitats, plants are under significant threat of various pathogens (like fungi, bacteria, viruses, and nematodes), which mostly succeed in invading plants that ultimately cause severe diseases. The interaction between plant and pathogen is a universal mechanism of signal activations, ultimately conferring resistance against pathogen attack or surrendering after pathogen attack. The communication between host and pathogen is the prime step to cause infection (Boyle and Finlay, 2003). For recognition, specific signals/ molecules (various kinds of polysaccharides and few glycoproteins) must be present on the plant cell surface, so the infection does not occur in case of their absence. Plants protect themselves from various stresses through their first line of defense, including physical barriers like cutin, stem bark, wax, and lignin. The physical barriers help protect plants from pathogen attacks and provide strength for their survival in life (Van Baarlen, 2007). In addition to barriers, different chemicals (like alkaloids, pyrethrins, cyanogenic glycosides, diterpenoids), secreted from plants, restrict the pathogen spread by killing them through lytic effects (Hammond-Kosack and Jones, 1996). The damage caused by a pathogen can be minimized by the organism's ability, termed resistance (Agrios, 1988). Plants showing resistance means pathogen failed to grow and spread its damaging effects in plants, usually through hypersensitive reaction. HR (hypersensitive reaction) leads to multiple outcomes like local cell death, restricting pathogen spread, and invasion (Bonas and Anckerveken, 1999). In addition, HR is responsible for activating multiple signal cascades, which involve various hormones and pathogen-related (PR) genes that play a vital role in developing resistance against various classes of pathogens (Benko-Iseppon *et al*., 2010). Acquired resistance is a phenomenon widely adopted by plants upon pathogen attacks, in which elevation in resistance has been observed. The biochemical activities induced in the host plant by pathogen attack are the last line of defense. The

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induced defense mechanism of plants includes two stages; pattern triggered immunity (PTI) and effector-triggered immunity (ETI), where PTI is induced when a pathogen is recognized through their pathogen-associated molecular pattern (PAMP) regions by pattern recognition receptors (PRR) of plants. In return, the activation of PTI activates many cell mechanisms, which enhance plants' resistance against attacking pathogens. The induced mechanisms include activation of mitogen-activated protein kinase (MAPK) cascades, increased reactive oxygen species, enhancement in the transcription of specific genes, which have a role in resistance induction in the plant. ETI activation is directed with plant R genes and linked with plant HR (Mandadi and Scholthof, 2013).

Pathogens easily break the resistance in plants due to their various receptors and effector responses. Plants have evolved multiple mechanisms to defend themselves from pathogen attack and ultimate damage. One of the most effective and complex mechanisms is recognition and triggering an effective response against a pathogen (Bolton, 2009). Resistance gene analogs (RGAs) are among the most complex systems evolved by plants to defend themselves from pathogen invasions. Resistance gene analogs (RGAs) are candidates of *R* genes with conserved motifs and domains, potentially conferring resistance against pathogens. Resistance gene analogs can be categorized into NBS-LRR (nucleotide-binding site leucine-rich repeat) and TM-LRR (transmembrane leucine-rich repeat). NBS-LRR (NLR) belongs to the apoptotic ATPase (AP-ATPase or NB-ARC ATPase) family of STAND (Signal Transduction ATPase with numerous domains) P-loop NTPase. These NTPases are signal-generating bodies and act as switches in plant and animal defense mechanisms. In NBS-LRR, a central NB domain at N-terminus and LRR at the C terminus is present. In addition to these, a region of homolog is present in between these two domains, which is known as ARC, i.e., Apaf1(apoptotic protease activating factor 1), R proteins, and CED4 (*C.elegans* Death-4) domain (van der Biezen and Jones, 1998). Molecular characterization and functional analysis revealed that the ARC domain contains two structural units, i.e., ARC1 and ARC2, involved in variable functions (Rairdan and Moffett, 2006). Multiple conserved motifs, including P-loop (Walker A/ Walker B), Kinases (1,2,3), hydrophobic GLPL, and RNBS (Resistance Nucleotide Binding Site), are present in the NB-ARC domain. Three peptide motifs in NBS are crucial for nucleotide-binding in various ATP/GTP binding. These peptides include P-loop (also known as a kinase-1a motif or Walker A motif), kinase-2 (Walker B) motif, and kinase-3a motif. Kinase-1a motif forms a glycine-rich loop containing Lysine residue, which plays a role in the phosphate-binding of nucleotides. The kinase-2 motif consists of Aspartic acid (Asp), which helps coordinate metal ions required in phosphotransfer reactions. The third peptide motif kinase-3a has a purine binding role in nucleotides (Traut, 1994). NBS-LRR is further categorized into TIR-NBS-LRR (toll/interleukin receptor nucleotide-binding site leucine-rich repeat), also known as TNL, and CC-NBS-LRR (non-TNL/coiled-coil nucleotide-binding site leucine-rich repeat), known as CNL (Hammond-Kosack and Jones, 1997). In addition to these subclasses, a small subgroup of NLR, i.e., Resistance to Powdery Mildew (RPW8), has been identified in *Arabidopsis thaliana*. Its initial identification was based upon the allele conferring resistance against powdery mildew disease, but later studies showed its resistance ability against other fungal diseases (Wang *et al*., 2008).

Mungbean (*Vigna radiata* L. Wilczek) is greatly affected by various pathogen attacks that invariably resulted in decreased growth and production, which ultimately left a devastating impact on farmers' income and various countries' economies. Among various diseases, Cercospora leaf spot (CLS) is a significant threat to mungbean production caused by *Cercospora canescens*. CLS is not limited to only Pakistan, but it is prevalent in all tropical Asian countries and Malaysia, Thailand, Bangladesh, the Philippines, Indonesia, and India (Pandey *et al*., 2018). The disease was first reported in Delhi (India), and it is found in tropical areas of Asia and other countries. It can cause approximately 60-100% loss in yield. Approximately 61% grain yield loss has been recorded (Iqbal *et al*., 1995). The disease spread rate is relatively high in susceptible varieties, ultimately resulting in grain and pod size decrement and plant defoliation (Grewal *et al*., 1978). So, it is necessary to search for such sources that can sustainably control pathogen and disease occurrence without affecting crop production and nutritious values. Identification of resistant mungbean varieties and the presence of Resistance gene analogs are the best way for such a purpose, which will result in long-term sustainable control of pathogens. The present research study identifies and characterizes Resistance gene analogs under the Cercospora leaf spot (CLS) challenge in *Vigna radiata*.

MATERIALS AND METHODS

***Mining of genic regions encoding the NBS-LRR domain in Vigna radiata*:** The genome of *Vigna radiata* was accessed through the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), and its available protein sequences were probed, using InterProScan V. 5.27 (https://www.ebi.ac.uk/interpro), for searching NBS-LRR domain.

Subcellular localization and Nuclear Localization Signals prediction: The subcellular localization of NBS domain-containing proteins was done using web server Target P1.1 http://www.cbs.dtu.dk/services/TargetP-1.1/index.php). For nuclear localization signals (NLS) NLStradamus program (http://www.moseslab.csb.utoronto.ca/NLStradamus/) was used under a two-state HMM static model by using posterior

and Viterbi methods with 6.0 cut off value. It was used to predict nuclear localization signals in probed and predicted NBS types.

***Structure pattern prediction*:** The structure pattern of NBS-LRR containing domain in *Vigna radiata* genome was made by Gene Structure Display Server (GSDS 2.0) (http://gsds.cbi.pku.edu.cn). GSDS is occasionally used to visualize the features of genes, including the position of introns, exons, and UTRs.

***Primer designing, synthesis, and screening*:** Primers were designed based on *in silico* identified NBS encoding genic regions of *Vigna radiata* and *R* genes (containing NBS-LRR domain) of *Vigna vexillata* and *Vigna unguiculata* retrieved from PRG (http://prgdb.crg.eu/). Additionally, NBS domain-based degenerate primers were designed and synthesized. The designed primers were screened for transcriptome profiling in PCR analysis, using DNA as a template.

***Mungbean Germplasm collection, inoculation, and screening*:** Mungbean germplasm was collected from NIAB (Nuclear Institute for Agriculture and Biology) and NARC (National Agricultural Research Centre) (Table 1). The plant material was grown in compost and maintained in the greenhouse of Fungal Molecular Biology (FMB) Laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad. Two groups of Mungbean genotypes were selected. One group consisted of two mungbean genotypes resistant to Cercospora leaf spot disease and a genotype susceptible to Cercospora leaf spot disease. These mungbean genotypes were inoculated with *Cercospora canescens*, a fungal pathogen of leaf spot in mungbean. The inoculation was done using hyphal suspensions on juvenile ten leaves (2 per plant) of mungbean. The leaves were then sprayed with distilled water and individually covered with polythene bags to maintain 100% RH (relative humidity) for 24 hrs. The inoculated plants were maintained in the greenhouse. Based on the collected germplasm screening, two groups (resistant and susceptible) of mungbean genotypes were selected for transcript profiling. The selected genotypes were NM92 and NM 98 (Resistant) and NCM251-4 (Susceptible).

***RNA isolation and cDNA synthesis*:** Total RNA from resistant and susceptible genotypes of mungbean under Cercospora leaf spot disease challenge was extracted. The extracted RNA was treated with a Rapidout DNA Removal kit to remove genomic DNA, cDNA was synthesized, and rt-PCR (reverse transcriptase-PCR) was performed. Differentially expressed DNA fragments amplified only in resistant mungbean genotypes and not shown any amplification in susceptible genotype or control were sequenced and *in silico* characterized.

***Homology and Protein motif search of identified RGAs*:** The DNA sequences of resistance gene analogs (RGAs) identified in mungbean under Cercospora leaf spot challenge was lodged into the BLASTX program (Translated Basic Local Alignment Search Tool) for their comparison as a query with non-redundant protein sequences in the GenBank database. The identified differentially expressed RGAs sequences were submitted to National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) database for the acquisition of GenBank accession number. The protein sequences of identified RGAs were predicted through the ExPASY translate tool (https://web.expasy.org/translate/), a SIB bioinformatics resource portal. The homology confirmation for deduced protein sequences of identified RGAs was done through Position-Specific Iterated BLAST (PSI-Blast).

***Alignment analysis*:** The translated protein sequences of identified *V. radiata* RGAs were aligned with protein sequences of known RGAs of other plant species. The

Table 1. The list of mungbean collected from NIAB (Nuclear Institute for Agriculture and Biology) and NARC (National Agricultural Research Centre) .

Sr.	Mungbean genotypes	Pedigree	Institute	Collected from
1	NM 98	NM 20-21 × VC 1482E	NIAB	NIAB
2	NM 92	NM 36 × VC 2768B	NIAB	NIAB
3	NM 51	VAR 6601 × VC 1973A/100 Gy	NIAB	NIAB
4	NM 54	VAR 6601 × VC 1973A/100 Gy	NIAB	NIAB
5	Ramzan Mung	VC 1482C × NM 92	NIFA, Peshawar	NIAB
6	Kabuli Mung	Wild Selection	Afghanistan	NIAB
7	AZRI Mung 2006	Line No. 1 × NM-96	AZRI, Bhakkar	NIAB
8	AZRI Mung 2018	NM-98 × Ramzan Mung	AZRI, Bhakkar	NIAB
9	NM 2016	VC 1482E × NM 20-21	NIAB	NIAB
10	Karak Mung-1	Local selection	ARS, Ahmad wala, Karak, KPK	NIAB
11	Tashqand Mung	Wild Selection	Tashqand	NIAB
12	BM 2017 (Bahawalpur Mung-17)		RARI, Bahawalpur	NIAB
13	NM 2011	VC 1482E × NM 20-21	NIAB	NIAB
14	VC-686	Exotic line	World Vegetable Centre	NIAB
15	NCM 255-8	----------	NARC	NARC
16	NCM 251-4	----------	NARC	NARC

alignment was made through MAFFT (Multiple Alignment using Fast Fourier Transform) tools, and results were visualized through the Jalview program.

***Domain identification*:** Translated protein sequences of identified RGAs of mungbean, CD-search, and CDART (Conserved Domain Architecture Retrieval Tool) were used. The structural motifs of these proteins were searched through the ScanProsite web tool.

***Computation of Physiochemical properties*:** The deduced protein sequences of identified *V. radiata* RGAs were computed through the online ProtParam tool. It helps in computing the physical and chemical properties of particular proteins.

***Subcellular localization*:** The subcellular localization was computed through CELLO v.2.5 (http://cello.life.nctu.edu.tw/). It is an SMV (Support Vector machine) classification system used to classify linear and non-linear data.

***Functional analyses of identified RGAs*:** The classification based on *in silico* functional analyses of translated protein sequences of identified RGAs was done through ProtoNet (http://www.protonet.cs.huji.ac.il/) displays automated hierarchical classification of protein sequences and through CATH database (https://www.cathdb.info/). MOTIF server (https://www.genome.jp/tools/motif/) was computed to identify the motifs present in translated protein sequences of identified *V. radiata* RGAs. The prediction of the protein-ligand binding site was computed using the COACH server (https://zhanglab.ccmb.med.umich.edu/COACH/), active site prediction of protein sequences through I-TASSER suit (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) and gene ontology (GO) using COFACTOR server (https://zhanglab.ccmb.med.umich.edu/COFACTOR/).

RESULTS

***In silico mining of genic regions encoding the NBS-LRR domain in Vigna radiata*:** Mungbean (*Vigna radiata*) is a diploid (2n=22) self-pollinated legume crop. Its estimated genome size is 494 to 579 Mb (https://plants.ensembl.org/). The genome of mungbean (*Vigna radiata*) was accessed from the NCBI database (https://www.ncbi.nlm.nih.gov/). A total of 49475 proteins were probed from the NCBI database. InterProScan V. 5.27 was used to find conserved domains of NBS-LRR class of *R* genes in the mungbean. The InterProScan annotation-based analysis showed that 695 proteins contained NBS domains and were classified as N (NBS), NL (NBS-LRR), TN (TIR-NBS), TNL (TIR-NBS-LRR), and RNL (RPW8-NBS-LRR). Among these, 329 belong to the N group, 267 to NL, 84 to TNL, 10 to TN, and 5 to the RNL group. Although CNLs and TNLs are present in all dicot plants, however, some dicots showed a higher number of TNL genes than CNLs, e.g., in case of *Arabidopsis thaliana*, *B. rapa*, *A. lyrate*, and *Glycine max* possess approximately sixfold more TNL genes as compared to CNLs (Kang *et al*., 2012; Yu *et al*., 2014). *Vigna radiata* is a dicotyledonous plant, and it completely lacks the CNL domain in the NBS-LRR family. The subcellular localization was done using an online Target P1.1 server (http://www.cbs.dtu.dk/services/TargetP-1.1/index.php). It helps in predicting the subcellular location of proteins assigned by the presence of N-terminal pre-sequences, which localize them to mitochondria (mTP signal peptide), secretory pathway (SP signal peptide), chloroplast (cTP signal peptide), and remaining to other locations in the cell. The analysis predicted 75 NBS proteins with mTP signals, 124 with SP signals, 101 with cTP signals, while 394 with signals for other subcellular locations. Thirty-one Ns, twenty-seven NLs, eight TNLs, and eight TNs were predicted to have putative nuclear localization signals (NLSs) through the NLStradamus program (Table 2).

The structure pattern of predicted NBS genes was explored by Gene Structure Display Server (GSDS 2.0). The sequences of NBS (N) and NBS-LRR (NL) groups displayed single gene architecture having UTRs with two CD regions and UTRs with thirteen CD regions, respectively. However, the number of CD regions for TIR-NBS-LRR (TNL) ranged from 1-3. Additionally, RPW8-NBS-LRR (RNL) and TIR-NBS (TN) contained a single exon (Fig. 1).

Primers were designed based on *in silico* identified NBS encoding genic regions. Keeping in view the gene structure display of predicted five groups of NBS types in mungbean (Fig.1), the conserved sequences of the RPW8-NBS-LRR (RNL) group were used for primer designing to identify resistance gene analogs (RGAs) from *Vigna radiata*. Furthermore, the *R* genes of its closely related species, *Vigna vexillata*, and *Vigna unguiculata*, were also used for primer designing as no *R* genes were reported in *Vigna radiata*. Additionally, NBS domain-based degenerate primers were designed and synthesized. Primers were designed through PrimerQuest Tool and were analyzed using OligoAnalyzer Tool, provided by Integrated DNA Technologies, Inc. [US] (Table 3). The primers were screened and selected in genomic analysis. The DNA of selected mungbean varieties was subjected to PCR analysis using designed primers. The primers that had shown amplification were selected for transcriptome profiling under Cercospora leaf spot disease.

***Transcriptome profiling of mungbean genotypes under Cercospora canescens challenge*:** Mungbean germplasm was inoculated with a characterized isolate of Cercospora canescens. Total RNA from mungbean genotypes, NM92, NM98, and NCM 251-4 under Cercospora leaf spot (CLS) disease challenge was extracted, and cDNA was synthesized. Reverse transcriptase PCR (rt-PCR) analysis was performed to recover putative resistance gene sequences or resistance gene analogs (RGAs, candidate R genes) expressed under Cercospora leaf spot stress.

Table 2. A list of NBS genes on mungbean (*Vigna radiata*) with Nuclear Localization Signals (NLS)

NBS TYPE	GenBank ID	Nuclear Localization Signal (NLS)
NBS	XP_022634232.1	235 - KARRKAKK – 242
	XP_014518785.1	235 - KARRKAKK – 242
	XP_022640519.1	32 - LKKMRDRKTIPRLRISKANRKPKLE – 56
	XP_014492173.1	3 - KKKEKKVNVSGKPKH – 17
		28 - KKERRSAGTVRR – 39
	XP_014492127.1	47 - RSKKPKKKYKPAAHRGFMARAKGIPALELYRLAQKKKRK – 85
	XP_014490178.1	390 - RKRKRQ – 395
		402 - KDREASLRSKKKARQ – 416
	XP_014489990.1	658 - KAPKVKAKSKMSKAEKEARKKQKMQAFQAAKQKSKGVKNSKRW – 700
	XP_014523081.1	26 - SRRKKKAREDARVK – 39
		735 - KDKNNKKLGKKKGPKT – 750
	XP_014523080.1	240 - RVWRRKDERKKKH – 252
		717 - EKEKNNRKNGKQKKNRKIGKEKKR – 740
	XP_014523000.1	83 - RARHLRDAARIAPEPRKKARGAAVAEAEKERAKKEKK - 119
		556 - IKSGKASPVKSMKKGIKVSKSG - 577
	XP_014520810.1	467 - RRGRAGRA - 474
	XP_014518712.1	124 - DRLKLERRKRKEER - 137
	XP_014516749.1	71 - GRKKKSK - 77
	XP_014514047.1	412 - RRRL - 415
	XP_014513606.1	263 - KERDEDRRKRKERKAA - 278
	XP_014513574.1	42 - RKNKKNKK - 49
	XP_014513375.1	686 - RRKLPRLLGFGPFRRDPNRRKLRRVKYYIGQKIKKIKQRRKDG - 728
	XP_014511906.1	116 - KAAQKKK - 122
	XP_014511905.1	122 - KAAQKKGK - 129
	XP_014510903.1	10 - RKKNKKLRKKNNSSSSSVSAKVAAVIAAKKRRKAGKRR - 47
	XP_014510851.1	3 - KKSKKSKSKRVSLKKKYKVIRKVKEHNKKKAKEAKKLRLSGRKKVEKDP - 51
	XP_014510314.1	550 - RKRSFRNKLKKRGTNK - 565
	XP_014509969.1	217 - KLKGKGGNGNGNGKKKEAVVAAKAEPKKVVKK - 248
	XP_014504006.1	9 - PRKRASKNSTPKKNTTPKKNSTPKRKSTPNKTPNKTK – 45
		997 - NRRKRRKK – 1004
	XP_014502397.1	111 - NIAKREKNK - 119
	XP_014502298.1	468 - KAKERKNQKLKMLAEKGLLKKNKRRKKSKE – 497
		519 - KTKSAKRKRFVNEKK - 533
	XP_014501161.1	2282 - KSKS - 2285
	XP_014499997.1	571 - QAKKRKRFERKTGVSKKRKVGSVTGRRP - 598
	XP_014497304.1	411 - LKSKPK - 416
	XP_014511273.1	309 - ERRKAKSREAAARQVRETVQARERWKIAKDIAKKGKGGGLG - 349
	XP_014522341.1	314 - RKTSKKPELKGFGSKHSDSAIPPKGKKKDK - 343
NBS-LRR	XP_014515322.1	39 - KVKRLREIK - 47
	XP_014515321.1	39 - KVKRLREIK - 47
	XP_022642993.1	210 - K - 210
	XP_022642239.1	61 - RDRIRKR - 67
	XP_022642038.1	37 - KKKVKRLG - 44
	XP_022642037.1	38 - KKVKR - 42
	XP_014515315.2	112 - RKAK - 115
	XP_014496898.2	799 - KGKKRKHLKN - 808
	XP_022635793.1	610 - RVSSKIWKLKRLRRL - 624
	XP_022635607.1	246 - RIRKKLMK - 253
	XP_022635605.1	247 - RKRLKK - 252
	XP_014490936.2	196 - KVKAKRKPSQEKTPSPPKKRR – 216
		196 - KVKAKRKPSQEKTPSPPKKRRI - 217
	XP_014518510.1	61 - RDRIRKR – 67
	XP_014511410.1	845 - KKMKRKTKKKNKTIQS – 860
		845 - KKMKRKTKKKNK - 856
	XP_014508891.1	27 - GRKNKRSKR - 35
	XP_014508890.1	27 - GRKNKRSKR - 35
	XP_014508889.1	27 - GRKNKRSKR - 35
	XP_014508888.1	27 - GRKNKRSKR - 35
	XP_014508887.1	27 - GRKNKRSKR - 35
	XP_014508886.1	27 - GRKNKRSKR - 35
	XP_014496900.1	610 - RVSSKIWKLKRLRRL - 624
	XP_014522609.1	251 - KRGK - 254
	XP_014522609.1	251 - KRGK - 254
	XP_014502954.1	275 - LKEKLKG - 281
	XP_022642993.1	210 - K - 210
	XP_022642038.1	37 - KKKVKRLG - 44
	XP_022642037.1	38 - KKVKR - 42
TIR-NBS	XP_022631468.1	388 - RPRRKS – 393
		440 - HPRLKRGKYAKRKNG - 454
	XP_022631467.1	388 - RPRRKS - 393
		440 - HPRLKRGKYAKRKNG - 454
	XP_022631466.1	388 - RPRRKS – 393
		440 - HPRLKRGKYAKRKNG - 454

NBS TYPE	GenBank ID	Nuclear Localization Signal (NLS)
	XP_022631465.1	388 - RPRRKS – 393
		440 - HPRLKRGKYAKRKNG - 454
	XP_022631464.1	388 - RPRRKS – 393
		440 - HPRLKRGKYAKRKNG - 454
	XP_022640079.1	134 - DKRSKKGRNKR – 144
		439 - RRSRLKRGGKYTRRKKGM - 456
	XP_022640078.1	134 - DKRSKKGRNKR – 144
		439 - RRSRLKRGGKYTRRKKGM - 456
	XP_014519514.1	388 - RPRRKS – 393
		440 - HPRLKRGKYAKRKNG - 45
TIR-NBS-LRR	XP_014516511.1	1015 - KKEPK - 1019
	XP_014516510.1	1015 - KKEPK - 1019
	XP_014516508.1	1015 - KKEPK - 1019
	XP_014516507.1	1015 - KKEPK - 1019
	XP_014516506.1	1015 - KKEPK - 1019
	XP_014515506.1	1042 - KAKKPKKK - 1049
	XP_014515505.1	951 - KKPK - 954
	XP_014502892.1	1073 – GRRGKRRKLHH-1164

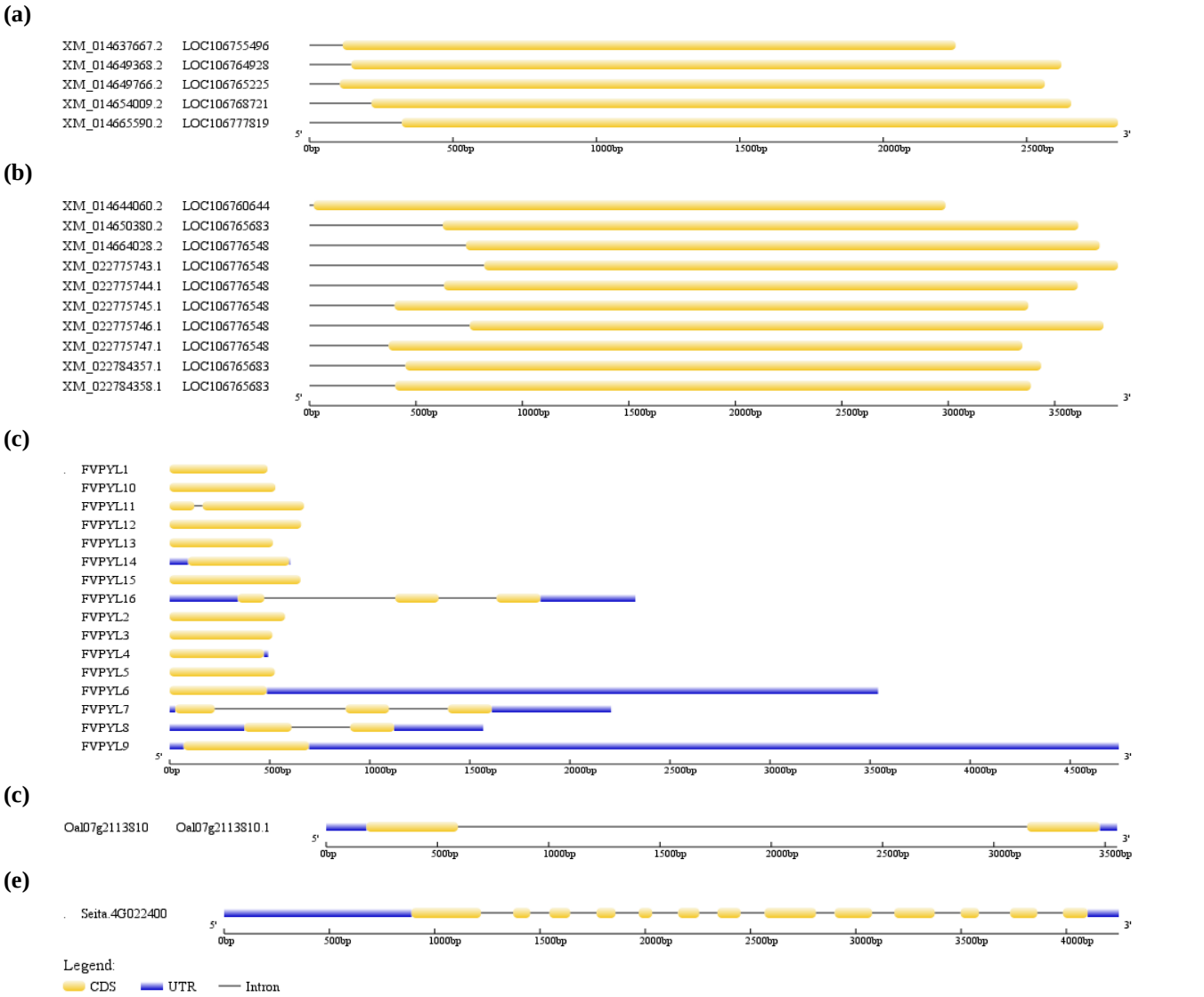

Figure 1. Gene structure of the coding sequence of NBS genes in mungbean (*Vigna radiata*): (a) RPW8-NBS-LRR (RNL) genes, (b) TIR-NBS (TN) genes, (c) TIR-NBS-LRR (TNL) genes, (d) NBS (N) genes, (e) NBS-LRR (NL) genes.

Table 3. List of primers designed from conserved domains of NBS-LRR class

Sr#	Primer	Primer Sequence (5' to 3' direction)	Amplicon size
1	RNLv1-F	CTAGAAGTGTATAATTTGATCGCTGATG	~ 241
	RNLv1-R	GTAACTGCTTGCACTTGTCAAC	
2	RNLv2-F	GGTGTGGGAAAGACAACACT	~ 304
	RNLv2-R	TGCCAGGACCAAACCAATC	
3	RNLv3-F	GCCCAATGGGTGTACAATGA	~ 343
	RNLv3-R	AGGTGTGAACAGTTTGGACTAC	
4	RNLv4-F	CAACACTCGCTTTAGCACTTTAT	~ 245
	RNLv4-R	CTCGTACCTGTCAACGTCATC	
5	RNLa1-F	GGC GGC GTC GGC AAG ACG AC	~ 480
	RNLb1-R	GAG GGC TAA AGG AAG GCC	
6	RNLa2-F	GGAGGA GTA GGA AAG ACG AC	~ 540
	RNLb2-R	CAG CGC CAG CGG CAG ACC	
7	RGMB1-F	CAACACTCGCTTTAGCACTTTATAATTTG	~316
	RGMB1-R	CCTCTAGTTGTAATGATGATCCTGCTGCC	
8	RGMB2-F	TTGGAAAAACAACACTTTCTCTAGAAG	~323
	RGMB2-R	CCTAGTTGTGATGATTATTTTGCTGCC	
9	RGMB3-F	GAATGGGTGGTGTGGGAAAGACAAC	~328
	RGMB3-R	GTGACTATGATTCTGCTGCCAGGAC	
10	RGMB4-F	TGGGAGGGGTGGGCAAAACTAC	~ 443
	RGMB4-R	GGTGGGGTGGGGAAGACACGC	
11	RGMB5-F	CATTGCAGCCCACACATTATC	~ 428
	RGMB5-R	CCGGCAATTCCTCCATATCA	
12	RGMB6-F	GGTTCTCCCTTGGCACTTATAG	~ 717
	RGMB6-R	CTCCAACTCAGCACGATTGA	
13	RGMB7-F	GCTAGGCAGATAGATGGTCTTAAC	~ 834
	RGMB7-R	GGATGCAGAGTGGCGAAATA	

The total reaction volume was 25 µl comprised of template DNA (50ng/µl), dNTPs (12mM), 10µM each forward and reverse primers,10X Taq buffer (2.5µl), Taq DNA polymerase (0.25µl) and nuclease-free water. The thermal profile for the amplification was an initial denaturation at 96°C for 5mins, followed by 35 cycles comprised of denaturation at 94°C for 45 sec, annealing at 55±2°C for 60 sec, extension at 72°C for 60 sec and final extension at 72°C for 10 minutes.

We got amplification in NM 92, NM 98, including NCM251-4 (susceptible genotype) by these primers in genomic analysis. However, in transcriptomic analysis, we did not get the amplification in NCM251-4 (susceptible genotype). That showed the primers amplify disease resistance-related genes or gene sequences expressed in resistant genotypes but not in susceptible genotypes. The primers did not show amplification in the NCM251-4 (susceptible genotype) transcriptome, but it showed amplification in its genome. It indicates some negative regulators in susceptible genotypes that may contribute to suppressing the expression of resistance genes.

Differentially expressed DNA fragments, which were amplified through reverse transcriptase PCR (rt-PCR) analysis (Fig. 2) only in CLS resistant genotypes, were eluted from the gel using FavorPrep gel purification mini kit (FAVORGEN, BIOTECH CORP., Taiwan) and directly sequenced by Eurofins DNA sequencing services, USA.

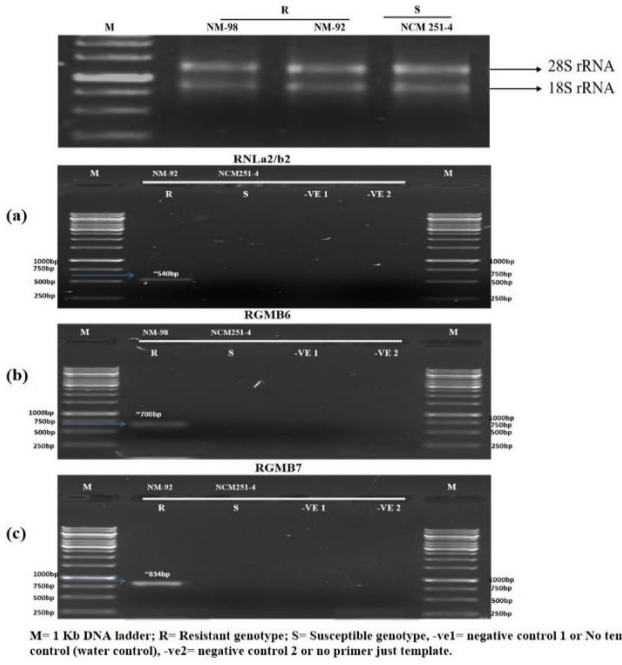

Figure 2. Reverse transcriptase PCR (rt-PCR) analysis of resistant mungbean genotypes (NM-92, NM-98) and susceptible mungbean genotype (NCM251-4) to recover putative disease resistance gene sequences against Cercospora Leaf Spot: (a) MB-ClsRCaG1 (b) MB-ClsRCaG2 (c) MB-ClsRCaG3.

The generated sequences were trimmed using BioEdit software version 7.2. These high-quality trimmed sequences were then subjected to BLAST (Basic Local Alignment Search Tool) for homology search to compare as a query with non-redundant protein sequences in the GenBank database. These generated sequences were translated into protein sequences using the ExPASy translate tool. The homology was searched through Position-Specific Iterated BLAST (PSI-Blast), which showed a 100% homology with disease resistance protein of *Vigna radiata* var. radiata. These sequences were submitted to GenBank, and assigned accession numbers were MT886216 (MB-ClsRCaG1), MT919519 (MB-ClsRCaG2), and MT919520 (MB-ClsRCaG3).

The conserved domain identification of translated protein sequences of identified *V. radiata* RGAs (MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3) was made through CD search and CDART (Conserved Domain Architecture Retrieval Tool), which revealed the presence of RPW8 and NB-ARC domains. RPW8 (Resistant to Powdery Mildew 8) is a resistance-conferring domain that can restrict various types of fungal pathogenic infection through a hypersensitive response (Zhao *et al*., 2021). NB-ARC belongs to the functional ATPase domain that plays a significant role in activating R protein (Van Ooijen et al., 2008). The presence of these two iconic resistance-inducing domains exhibits disease resistance attribution of identified *V. radiata* RGAs.

MAFFT analysis of sequence alignment of identified *V. radiata* RGAs with known plants' RGAs showed conserved NBS domains. Jalview alignment results displayed P-loop's presence in MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3. However, the RNBSA-non TIR and Kinase 2 motifs were present only in MB-ClsRCaG1 (Fig. 3). All NBS possess an ordered motif backbone of P-loop/ Kinase/ RNBS/ GLPLA surrounding the NBS domain. P-loop and Kinase 2 are highly conserved motifs as they are involved in nucleotide triphosphate binding. The P-loop motif directly interacts with NTP phosphate, while Kinase-2 contains aspartate crucial for the phosphotransfer process (Traut, 1994). The structural motifs of MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3 were identified using the ScanProsite web tool (Table 4). The results showed the presence of significant conserved motifs along with their signature sequences, including P-loop (GLGGSGKS), protein kinase (TVR/ SQK/ SGK/ SRK), N-myristoylation site (GSVLTV/ GTGGSG/ GSGKST), Tyrosine kinase (RLFEHNGY), cAMP- and cGMP-dependent protein kinase (RKLS, KKLS) and Casein Kinase II phosphorylation site (SILE/ SSQE/ SPPE/ SDEE).

Table 4. Table: ScanProsite, CD search, and CDART showed the presence of conserved domains and motifs along with their signature sequence in recovered RGAs of *V. radiata*

Name	Motifs	Signature Sequence	Identified Conserved Domain through CDART and CD search
MB-ClsRCaG1	Casein kinase II phosphorylation site	SSQE, SPPE, SDEE, SLAE, TIVE	RPW8 and NB-ARC
	N- myristoylation site	GSVLTV, GTGGSG, GSGKST	
	ATP/GTP- binding site motif A (P-Loop)	GTGGSGKS	
	Protein kinase C phosphorylation site	SGK	
	Tyrosine kinase phosphorylation site 1	RLFEHNGY	
MB-ClsRCaG2	RPW8	DALVGH, RIAGLC	RPW8
	Protein kinase C phosphorylation site	TVR, SQK, SGK	
	Casein Kinase II phosphorylation site	SILE, SSQE, SPPE, SDEE	
	N-myristoylation site	GSVLTV, GTGGSG, GSGKST	
	ATP/GTP binding site motif A (P-loop)	GTGGSGKS	
MB-ClsRCaG3	Protein kinase C phosphorylation site	SGK, SRK	------
	cAMP- and cGMP-dependent protein kinase	RKLS, KKLS	
	Casein kinase II phosphorylation site	SISD	
	N- myristoylation site	GLGGSG, GSGKSR	
	ATP/GTP- binding site motif A (P-Loop)	GLGGSGKS	

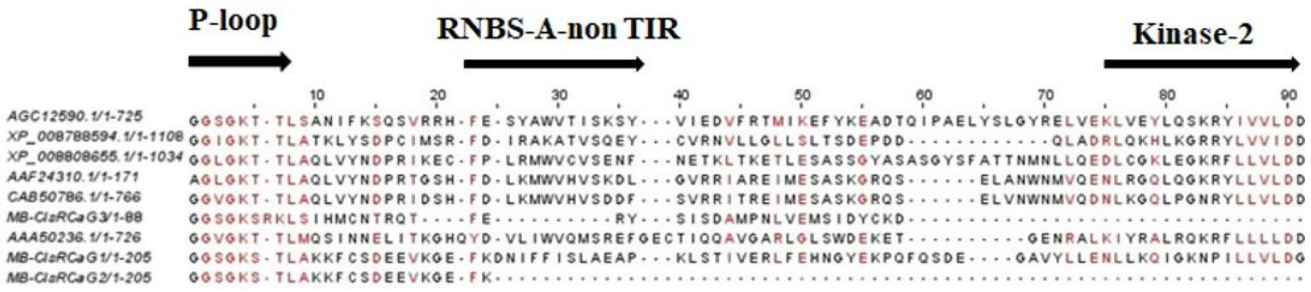

Figure 3. MAFFT analysis of sequence alignment to identify conserved motif in identified *V. radiata* RGAs

The consensus sequences of these conserved motifs have been reported in various studies (Traut, 1994; Hammond-Kosack and Jones, 1997; Meyers *et al*., 1999; Tameling *et al*., 2002). Protein kinases are involved in the phosphorylation and dephosphorylation of various proteins during plant stress conditions and significantly impact proteins' activation in multiple signaling cascades (Dai *et al*., 2013). Casein Kinase II is also a protein kinase involved in various cellular processes and stress responses (Ahmed *et al*., 2002; Mulekar and Huq, 2014). N-terminal myristoylation protein is involved in various cellular pathways and has a significant role in cellular apoptosis and signal transduction. It helps in the stability of proteins and their interaction with hydrophobic proteins (Olsen and Kaarsholm, 2000). Tyrosine kinase is a significant regulator of signal transduction regulation and involved in plant growth and defense responses against biotic and abiotic factors (Miyamoto *et al*., 2019). However, cAMP- and cGMP-dependent protein kinase are significantly involved in various physiological functions and signal transduction (Isner *et al*., 2012).

ProtoNet tools classified the identified MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3 in the NB-ARC cluster, sequence of MB-ClsRCaG1 and MB-ClsRCaG2 is similar to VRP1-1 protein while the sequence of MB-ClsRCaG3 is most similar to Disease resistance protein-like protein MsR1. VRP1-1 is a disease resistance protein, belongs NBS-LRR class, and contains P-loop, NB-ARC, and RPW8 domain. While MsR1 (macrophage scavenger receptor 1) with a significant role in regulating immune homeostasis (Tang *et al*., 2018).

The physicochemical properties of identified RGAs were computed through the online ProtParam tool. The number of amino acids of the translated protein sequence of identified RGA, MB-ClsRCaG1, was computed as 205 with 22932.55 molecular weight (MV) and 5.98 theoretical PI (isoelectric point). The PI value indicated the acidic nature. The molecular formula predicted as $C_{1035}H_{1646}N_{268}O_{302}S_8$ containing 3259 atoms, while the instability index (II) was computed as 31.07, which declared MB-ClsRCaG1 stable as proteins with instability index lower than 40 is categorized as stable protein. The aliphatic index and GRAVY (Grand average of hydropathicity) of MB-ClsRCaG1 were computed as 101.22 and -0.145. The Aliphatic index value is related to the thermal stability of protein, i.e., the higher the Aliphatic index of a protein, the more is its thermal stability. The aliphatic index of MB-ClsRCaG1 protein indicated its thermal stability. The hydrophobicity value of a protein is determined with GRAVY (Grand average of hydropathicity index). The positive GRAVY index indicates that protein is hydrophobic, and the negative value represents the protein's hydrophilic nature. In this case, the MB-ClsRCaG1 protein is hydrophilic.

Similarly, the translated protein sequence of MB-ClsRCaG2 contains 205 amino acids with 23141.84 molecular weight (MV) and 7.79 theoretical PI (isoelectric point). According to the PI value, the protein is neutral. The predicted molecular formula $C_{1025}H_{1669}N_{283}O_{304}S_{10}$ contains 3291 atoms, while the instability index (II) was computed as 30.42 (instability index less than 40), which declared the MB-ClsRCaG2 to be stable. The aliphatic index and GRAVY (Grand average of hydropathicity) of MB-ClsRCaG2 were computed at 96.93 and -0.354, respectively. A high aliphatic index indicates thermal stability, while a negative GRAVY value makes it a hydrophilic protein. In MB-ClsRCaG3, the protein contains 88 amino acids with 9793.62 molecular weight (MV) and 9.15 theoretical PI (isoelectric point), indicating its alkaline nature. The molecular formula is predicted as $C_{429}H_{726}N_{118}O_{127}S_7$ containing 1407 atoms, while the instability index (II) was computed as 34.77, i.e., less than 40 declared MB-ClsRCaG3 to be stable. The aliphatic index and GRAVY (Grand average of hydropathicity) of MB-ClsRCaG3 were computed as 109.55 and -0.024, respectively, which means it is a thermally stable hydrophilic nature protein.

CELLO v.2.5 (http://cello.life.nctu.edu.tw/) predicted the localization of MB-ClsRCaG1 in the cytoplasm with significant reliability value 4.346, MB-ClsRCaG2 in the cytoplasm with reliability 4.102 and for MB-ClsRCaG3 in nuclear and mitochondria with significant reliability of 1.345 and 1.531, respectively. The R proteins might reside in the cytoplasm or nucleus and sometimes in both locations. However, these proteins can also be localized in inter and intracellular membranes (Berkey *et al*., 2017). MRP-1 (multidrug resistance-associated protein 1) is expressed in mitochondria (Roundhill *et al*., 2016). Approximately 450 PPRP (Pentatricopeptide repeat proteins) involved in stress responses are localized in mitochondria (Laluk *et al*., 2011).

The functional family predicted through the CATH database for MB-ClsRCaG1, MB-ClsRCaG2 and MB-ClsRCaG3 were probable disease resistance proteins. This analysis predicted the functional superfamily P-loop containing nucleotide triphosphate hydrolases superfamily for MB-ClsRCaG1, and MB-ClsRCaG2, while Ribonuclease inhibitor superfamily for MB-ClsRCaG3. Ribonuclease inhibitor (RI) structural units comprise leucine-rich residues that have an imperative role in protein-protein interaction during pathogen attack (Dickson *et al*., 2005). MOTIF server predicted NB-ARC and RPW8 in both MB-ClsRCaG1 and MB-ClsRCaG2 (Fig. 4), while no result was shown for MB-ClsRCaG3, that could be due to its short sequence length.

The active site residues of MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3 were predicted through the I-TASSER server. In MB-ClsRCaG1, the predicted active sites were Ser122 (S), Ser146 (S), Asp198 (D), and Gly199 (G). The predicted active sites for MB-ClsRCaG2 include His33 (H), Ser34 (S), Ile35 (I), Lys71 (K), and Asn75 (N). For MB-ClsRCaG3, the predicted active site residues were Glu11 (E), Leu13 (L), His37 (H), Asp63 (D), Ser35 (S), Ser85 (S), and Ser61 (S).

The protein-ligand binding sites predicted by the COACH database for MB-ClsRCaG1 were Lys98 (K) Leu101 (L), Val110 (V), and Leu111 (L). For MB-ClsRCaG2, the ligand-binding sites include Lys69 (K), Gly70 (G), Leu98 (L), Ile102 (I), and Try105 (Y). In the case of MB-ClsRCaG3, ligand binding sites were Leu12 (L), Lys14 (K), Ser35 (S), His37 (H), Ser61 (S), Asp63 (D), and Ser85 (S) (Fig. 5).

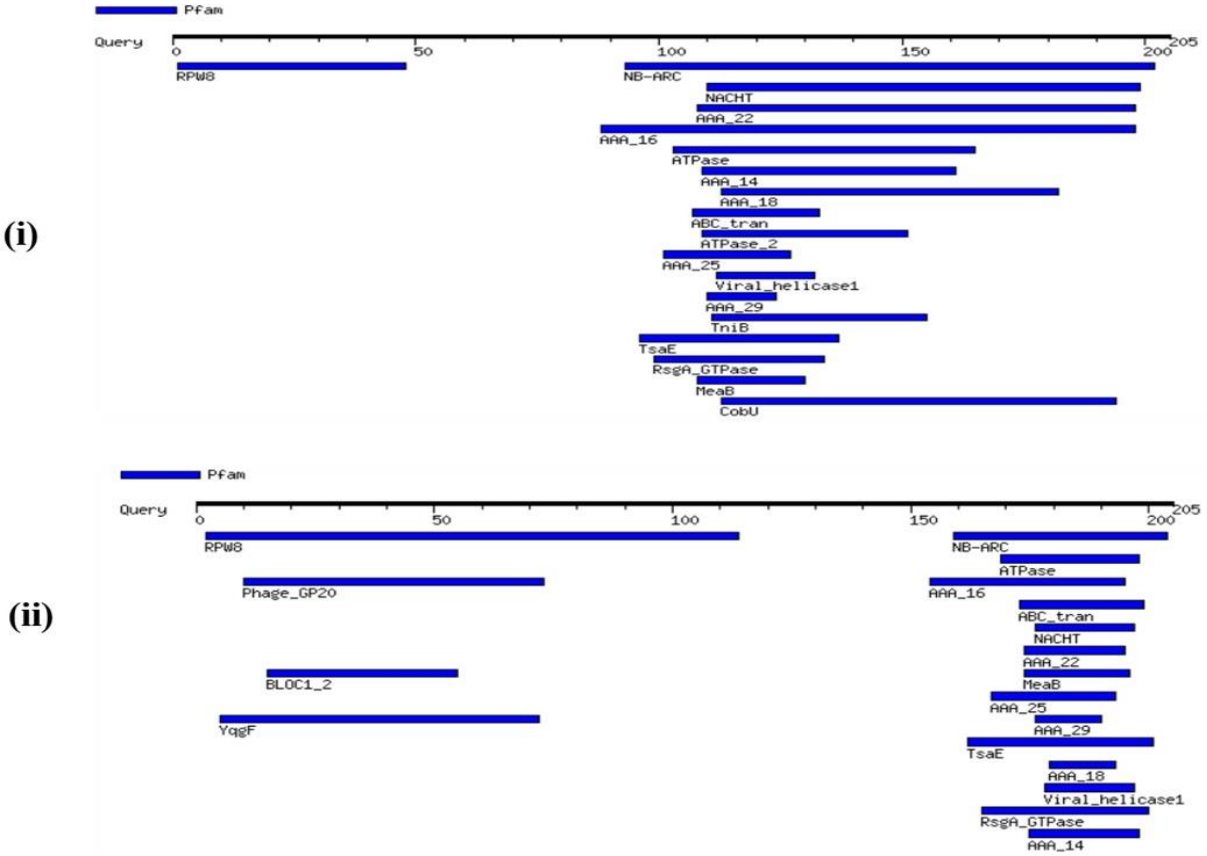

Figure 4. MOTIF server displayed conserved motif in (i) MB-ClsRCaG1 (ii) MB-ClsRCaG2

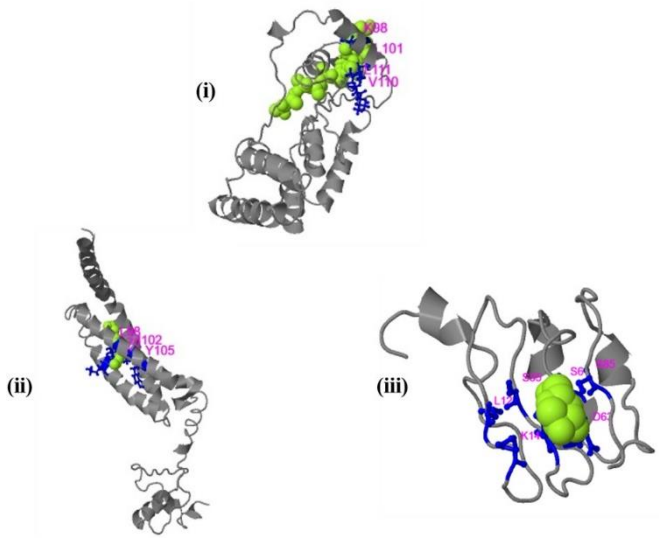

Figure 5. COACH database prediction of Protein-ligand binding sites in (i) MB-ClsRCaG1 (ii) MB-ClsRCaG2 and (iii) MB-ClsRCaG3.

Table 5. The biological process, cellular component, and molecular function of Identified putative RGAs

Identified putative RGAs	Biological Process (BP)	Cellular Component (CC)	Molecular Function (MF)
MB-ClsRCaG1	○ Regulation of Apoptotic process ○ Defense response ○ Embryonic morphogenesis ○ Cell death ○ Regulation of cell size ○ DNA replication ○ Cell division	○ Cytosol ○ Nucleus ○ Perinuclear region of cytoplasm ○ Mitochondrion ○ Golgi apparatus ○ Nucleolus	○ Domain binding ○ ATP binding ○ Nucleotide-binding ○ Protein binding ○ Caspase activator ○ Lyase activity ○ Catalytic activity
MB-ClsRCaG2	○ Metabolic process ○ AMP biosynthetic process ○ Protein tetramerization ○ Homeostasis ○ Cellular respiration ○ Catabolic process ○ Protein oligomerization	○ Cytoplasm ○ Mitochondria ○ Cytosol ○ Macromolecular complex ○ Intracellular membrane-bounded organelle	○ Lyase activity ○ Catalytic activity ○ Metal ion binding ○ Isomerase activity ○ Nucleotide-binding ○ Protein binding
MB-ClsRCaG3	○ Response to stress ○ Defense response to bacteria and other microbes ○ Regulation of cellular process ○ Regulation of protein kinase activity ○ Negative regulation of signal transduction	○ An intrinsic component of membrane ○ Membrane ○ Cell part ○ Plasma membrane ○ An integral component of membrane	○ Molecular function regulator ○ Protein binding

The gene ontology (GO) of MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3 was predicted using the COFACTOR server. The biological process, cellular component, and molecular function mainly represent their critical role in defense mechanisms. These proteins' GO showed their involvement in defense responses, apoptotic regulations, maintaining homeostasis, and cellular respiration (Table 5).

DISCUSSION

Resistance gene analogs (RGAs) are *R* gene candidates comprised of various conserved domains and motifs, which are imperative for conferring resistance against pathogens (Fujita *et al*., 2006; Yang *et al*., 2008). RGAs are categorized into NBS-LRR (nucleotide-binding site leucine-rich repeat) and TM-LRR (transmembrane leucine-rich repeat). NBS-LRR comprised of NB domain (at N terminus), LRR domain (at C terminus) and central ARC domain (van der Biezen and Jones, 1998). The mining of NBS-LRR genic regions in *Vigna radiata*, done through NCBI and InterProScan, showed the presence of NBS types, including N (NBS), NL (NBS-LRR), TN (TIR-NBS), TNL (TIR-NBS-LRR), and RNL (RPW8-NBS-LRR). Dicotyledonous plants either completely lack CNLs (CC-NBS-LRR) or contain a lesser number of CNLs as compared to TNLs as in the case of *Glycine max* (a dicot), which contains six-folds higher TNLs than CNLs (Yu *et al*., 2014; Lozano *et al*., 2015). *Vigna radiata*, as a dicotyledon, does not contain any CNL domain. The signal peptide prediction of probed NBS-LRR proteins revealed that many proteins were targeted to other subcellular locations (other than mitochondria and chloroplast), as various studies have previously suggested the localization of NBS-LRR proteins on the inner region of a membrane (Mackey *et al*., 2002; Axtell and Staskawicz, 2003). Moreover, some NBS-LRR proteins contain nuclear localization signals, as according to previous studies, RRS1, a disease resistance protein, showed its localization in the nucleus during pathogen attack (Deslandes *et al*., 2003). The structure pattern of probed NBS genes showed a single type of exon presence in RNL and TN. At the same time, CD regions were present in NL, TNL, and N. The single exon type in RNL was used for primer designing to identify RGA (s) in *Vigna radiata* under Cercospora leaf spot (CLS) challenge as RPW8 (Resistance to Powdery Mildew), a small subgroup of NLR, showed its resistance ability against many fungal diseases (Wang *et al*., 2008). Additionally, degenerate primers and conserved domains of *R* genes from closely related species of *Vigna radiata* were used for primer designing and synthesis. It has been reported in many studies in which the conserved domains of NBS-LRR from closely related species were taken into account to identify RGA (s) in closely related crops (Lee *et al*., 2003). Moreover, the conserved nature of NBS-LRR made it possible to use the *R* genes of diverse plants as degenerate primers to identify resistance genes in distant crop species (Totad *et al*., 2005). Three RGAs (MB-ClsRCaG1, MB-ClsRCaG2, and MB-ClsRCaG3) of *Vigna radiata* were identified, and their translated protein sequences showed a 100% homology match with disease resistance protein of *Vigna radiata* var. radiata. It confirmed that these are putative RGAs with disease resistance potential. MAFFT analysis showed the presence of P-loop, kinase 2, and RNBSA-non TIR in identified RGAs. Moreover, ScanProsite displayed the

presence of conserved motifs including P-loop, protein kinase, N-myristoylation site, Tyrosine kinase, cAMP- and cGMP-dependent protein kinase, and Casein Kinase II phosphorylation site. P-loop (also known as kinase-1a motif), kinase-2 (also called Walker B) motif, and kinase-3a motif of NBS have a significant role in nucleotide-binding in various ATP/GTP binding (Traut, 1994). The classification of identified RGAs of *Vigna radiata* through Protonet showed their similarity to VRP1-1 protein and MsR1 (macrophage scavenger receptor 1), former is a disease resistance protein, belong to NBS-LRR class and contain P-loop, NB-ARC, and RPW8 domain, while MsR1 play a significant role in regulating immune homeostasis (Tang *et al*., 2018). Ribonuclease inhibitor (RI) present in MB-ClsRCaG3 indicates its role in disease resistance as RI structural units comprise leucine-rich residues, which have an imperative role in protein-protein interaction during pathogen attack (Dickson *et al*., 2005). Regarding physiochemical properties, all three proteins of RGAs have a high aliphatic index as the aliphatic index value is related to the thermal stability of protein, i.e., the higher the Aliphatic index of a protein, the more is its thermal stability (Panda and Chandra, 2012). The proteins with an instability index lower than 40 are categorized as stable proteins, so the translated proteins of identified RGAs were considered stable as their index value was lower than 40. The hydrophobicity value of a protein is determined with GRAVY (Grand average oh hydropathicity index). The positive GRAVY index indicates that protein is hydrophobic, and the negative value represents the hydrophilic nature of the protein (Kyte and Doolittle, 1982). The GRAVY values of all translated proteins of RGAs have a negative value which indicates their hydrophilic nature. Subcellular localization holds significant importance in predicting the potential of RGAs. Various studies reported the localization of resistant genes in the cytoplasm or nucleus (Meier and Somers, 2011). In our case, putative RGAs showed their localization in the cytoplasm as well as in the nucleus. The resistance genes localized in the cytoplasm are involved in hypersensitive responses, while residing nuclear genes help acquire complete resistance against the pathogen (Bai *et al*., 2012).

***Conclusion*:** This study uncovered the genetics involved in the Cercospora leaf spot disease in *Vigna radiata*. Three resistance gene analogs (RGAs) were identified in NM 92, NM 98 (reported resistant genotypes), contributing to resistance against *Cercospora canescens* in the mungbean. These putative RGAs contained conserved domains (P-loop, Kinase-2) involved in disease resistance. Hence this study will help in long-term sustainable control for this fungal pathogen.

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REFERENCES

Agrios, G.N. 1988. Plant Pathology, 3rd Ed. Academic Press., USA.

Ahmed, K., D.A. Gerber and C. Cochet. 2002. Joining the cell survival squad: an emerging role for protein kinase CK2 Trends. Cell Biol. 12: 226-230.

Axtell, M.J. and B.J. Staskawicz. 2003. Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. Cell. 112: 369-377.

Bai, S., J. Liu, C. Chang, L. Zhang, T. Maekawa, Q. Wang, W. Xiao, Y. Liu, J. Chai, F.L. Takken and P. Schulze-Lefert. 2012. Structure-function analysis of barley NLR immune receptor MLA10 reveals its cell compartment specific activity in cell death and disease resistance. PLoS Pathog. 8:e1002752

Benko-Iseppon, A.M., S.L. Galdino, T. Calsa Júnior, E.A. Kido, A. Tossi, L.C. Belarmino and S. Crovella. 2010. Overview of plant antimicrobial peptides. Curr. Prot. Pept. Sci. 11:181-188.

Berkey, R., Y. Zhang, X. Ma, H. King, Q. Zhang, W. Wang and S. Xiao. 2017. Homologues of the RPW8 resistance protein are localized to the extrahaustorial membrane that is likely synthesized de novo. Plant Physiol. 173:600-613

Bolton, M. 2009. Primary metabolism and plant defense – Fuel for the fire. Mol. Plant. Microbe Interact. 22: 487-497.

Bonas, U. and G.V. Anckerveken. 1999. Gene-for-gene interactions: Bacterial avirulence proteins specify plant disease resistance. Curr. Opin. Plant Biol. 2:94-98.

Boyle, E.C. and B.B. Finlay. 2003. Bacterial pathogenesis: exploiting cellular adherence. Curr. Opin. Cell Biol. 15:633-9

Dai, M., Q. Xue, T. Mccray, K. Margavage, F. Chen, J.H. Lee, C.D. Nezames, L. Guo, W. Terzaghi, J. Wan, X.W. Deng and H. Wang. 2013. The PP6 phosphatase regulates ABI5 phosphorylation and abscisic acid signaling in Arabidopsis. Plant Cell 25:517-534.

Deslandes, L., J. Olivier, N. Peeters, D.X. Feng, M. Khounlotham, C. Boucher, I. Somssich, S. Genin and Y. Marco. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. 100: 8024–8029

Dickson, K.A., M.C. Haigis and R.T. Raines. 2005. Ribonuclease inhibitor: structure and function. Prog. Nucleic Acid Res. Mol. Biol. 80:349-374.

Fujita, M., Y. Fujita, Y. Noutoshi, F. Takahashi, Y. Narusaka, K. Yamaguchi-Shinozaki and K. Shinozaki. 2006.

Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr. Opin. Plant Biol. 9:436-442

Grewal, J.S. 1978. Diseases of mungbean in India. In: 1st International Mungbean Symposium AVRDC; Aug 16-19, 1978. Philippines. pp.165-168.

Hammond-Kosack, K.E. and J.D. Jones. 1996. Resistance gene-dependent plant defense responses. The Plant Cell. 8:1773

Hammond-Kosack, K.E. and J.D. Jones. 1997. Plant disease resistance genes. Plant Mol. Biol. 48:575-607.

Iqbal, S.M., A. Ghafoor, M. Bashir and B.A. Malik. 1995. Estimation of losses in yield components of mugbean due to Cersospora leaf spot. Pakistan J. Phytopathol. **7**: 80-81.

Isner, J.C., T. Nühse and F.J. Maathuis. 2012. The cyclic nucleotide cGMP is involved in plant hormone signalling and alters phosphorylation of Arabidopsis thaliana root proteins. J. Exp. Bot. 63:3199-3205

Kyte, J. and R.F. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. J. Mol Biol. 157:105-132

Kang, Y.J., K.H. Kim, S. Shim, M.Y. Yoon, S. Sun, M.Y. Kim, K. Van and S.H. Lee. 2012. Genome-wide mapping of NBS-LRR genes and their association with disease resistance in soybean. BMC Plant Biol. 12: 1-3.

Lee, S.Y., J.S. Seo, M. Rodriguez-Lanetty and D.H. Lee. 2003. Comparative analysis of superfamilies of NBS-encoding disease resistance gene analogs in cultivated and wild apple species. Mol. Genet. Genom. 269:101-108

Laluk, K., S. Abu Qamar and T. Mengiste. 2011. The Arabidopsis mitochondria-localized pentatricopeptide repeat protein PGN functions in defense against necrotrophic fungi and abiotic stress tolerance. Plant Physiol. 156:2053-2068

Lozano, R., M.T. Hamblin, S. Prochnik and J.L. Jannink. 2015. Identification and distribution of the NBS-LRR gene family in the Cassava genome. BMC Genom. 16:1-4

Mackey, D., B.F. Holt III, A. Wiig and J.L. Dangl. 2002. RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108: 743-754.

Meier, I. and D.E. Somers. 2011. Regulation of nucleocytoplasmic trafficking in plants. Curr. Opin. Plant Biol. 14:538-546

Mandadi, K.K. and K.B. Scholthof. 2013. Plant immune responses against viruses: how does a virus cause disease. The Plant Cell. 25:1489-1505

Meyers, B.C., A.W. Dickerman, R.W. Michelmore, S. Sivaramakrishnan, BW. Sobral and ND. Young. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J. 20: 317–332.

Miyamoto, T., T. Uemura, K. Nemoto, M. Daito, A. Nozawa, T. Sawasaki and G.I. Arimura. 2019. Tyrosine kinase-dependent defense responses against herbivory in Arabidopsis. Front. Plant Sci. 10:776.

Mulekar, J.J. and E. Huq. 2014. Expanding roles of protein kinase CK2 in regulating plant growth and development. J. Exp. Bot. 65:2883-2893

Olsen, H.B. and NC. Kaarsholm. 2000. Structural effects of protein lipidation as revealed by LysB29-myristoyl, des(B30) insulin. Biochemistry. 39:11893-11900.

Panda, S. and G. Chandra. 2012. Physicochemical characterization and functional analysis of some snake venom toxin proteins and related non-toxin proteins of other chordates. Bioinformation.

Pandey, A.K., R.R. Burlakoti, L. Kenyon and R.M. Nair. 2018. Perspectives and challenges for sustainable management of fungal diseases of mungbean [Vigna radiata (L.) R. Wilczek var. radiata]. A Rev. Front. Environ. Sci. 6:53

Rairdan, G.J. and P. Moffett. 2006. Distinct domains in the ARC region of the potato resistance protein Rx mediate LRR binding and inhibition of activation. The Plant Cell. 18:2082-2093.

Roundhill, E., D. Turnbull and S. Burchill. 2016. Localization of MRP-1 to the outer mitochondrial membrane by the chaperone protein HSP90β. The FASEB. J. 30:1712-23.

Traut, T.W. 1994. The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide-binding sites. In: ChristenE, P. and E. Hofmann (eds.), EJB Rev. Springer, Berlin, Heidelberg. Pp. 105-115.

Tameling, W.I., S.D. Elzinga, P.S. Darmin, J.H. Vossen, F.L. Takken, M.A. Haring and B.J. Cornelissen. 2002. The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. Plant Cell 14: 2929–2939.

Totad, A.S., B. Fakrudin and M.S. Kuruvinashetti MS. 2005. Isolation and characterization of resistance gene analogs (RGAs) from sorghum (Sorghum bicolor L. Moench). Euphytica. 143:179-88.

Tang, Y., H. Li, J. Li, Y. Liu, Y. Li, J. Zhou, J. Zhou, X. Lu, W. Zhao, J. Hou and X.Y. Wang. 2018. Macrophage scavenger receptor 1 contributes to pathogenesis of fulminant hepatitis via neutrophil-mediated complement activation. J. Hepatol. 68:733-43.

Van Baarlen, P., A. Van Belkum, R.C. Summerbell, P.W. Crous and B.P. Thomma. 2007. Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps. FEMS Microbiol. Rev. 31:239-277.

van der Biezen, E.A. and J.D. Jones. 1998. The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. Curr. Biol. 8:R226-R228

Van Ooijen, G., G. Mayr, M.M. Kasiem, M. Albrecht, B.J. Cornelissen and F.L. Takken. 2008. Structure–function analysis of the NB-ARC domain of plant disease resistance proteins. J. Exp. Bot. 59:1383-1397

Wang, W., A. Devoto, J.G. Turner and S. Xiao. 2008. Expression of the membrane-associated resistance protein RPW8 enhances basal defense against biotrophic pathogens. Mol. Plant microbe interact. 20:966-976

Yang, X.Y., X.W. Wang, X.B. Li, B.B. Zhang, Y.H. Xiao, D.M. Li, C.J. Xie and Y. Pei. 2008. Characterization and expression of an nsLTPs-like antimicrobial protein gene from motherwort (Leonurus japonicus). Plant Cell Rep. 27:759-766.

Yu, J., S. Tehrim, F. Zhang, C. Tong, J. Huang, X. Cheng, C. Dong, Y. Zhou, R. Qin, W. Hua and S. Liu. 2014. Genome-wide comparative analysis of NBS-encoding genes between Brassica species and Arabidopsis thaliana. BMC Genom. 15:1-8

Zhao, Z.X., Y.J. Xu, Y. Lei, Q. Li, J.Q. Zhao, Y. Li, J. Fan, S. Xiao and W.M. Wang. 2021. ANNEXIN 8 negatively regulates RPW8. 1-mediated cell death and disease resistance in Arabidopsis. J. Integr. Plant Biol. 63:378-392.