

# Molecular detection and characterization of disease resistance genes for bacterial blight in selected Indian soybean varieties

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## ABSTRACT

Soybean is an essential legume crop grown worldwide due to its high nutrient value and higher percentage of protein contain. Various pathogens and pests cause diseases which result in low yield of soybean crop. Bacterial blight and soybean mosaic are the two most important diseases of *Glycine max* which affect the crop production. Plants protect themselves from these attacks through various mechanisms, one of which involves *R* (resistance) gene which provides resistance to plant against various diseases for instance; *Rpg1b* gene (type of nucleotide-binding sites-leucine-rich repeats [NBS-LRR] genes) provides resistance against bacterial blight disease in soybean. *R* genes cloning is the most recent method for obtaining disease resistance legume varieties. The genome of Williams 82 cultivar of *G. max* is completely sequenced but very little is known about the genome of Indian soybean varieties. Thus, identification of *Rpg1b* gene analogs in Indian variety is beneficial in breeding programs of soybean. Genomic DNA was extracted from selected genotypes various Indian soybean cultivars using CTAB method, purified and amplified using primers designed for NBS and LRR regions of *Rpg1b* genes. Both primers showed amplification in selected cultivars which confirmed the presence of *Rpg1b* gene in them. After gene sequencing, comparison of these sequences was done with Williams 82 cultivar using box shade tool which showed variations among them. This study on *Rpg1b* gene in various Indian soybean cultivars will be beneficial for the development of *R* gene-based molecular markers linked to bacterial blight in soybean.

## 1. INTRODUCTION

Soybean (*Glycine max*) is an important crop globally due to its high percentage of proteins and oil contain [1,2]. Various food products are made from soybean seeds [3] and substantial efforts have been placed on increasing soybean yield in order to fulfill the requirement of larger population worldwide [4,5]. Pests, pathogens, and weeds cause detrimental effect resulting in low production of soybean crop [6]. Microorganisms such as fungi, viruses, bacteria, oomycetes, and nematodes contribute to economic damage every year [7]. The most important diseases of soybean are soybean rust, bacterial blight, and soybean mosaic which affect soybean production.

Plants need to protect themselves against attack from viruses, microbes, invertebrates, and even other plants [8]. It has been speculated that plants have two different types of disease resistance system called as basal defense and *R* gene involving defense [9-11]. The *R* genes are considered to be specificity determinants of the plant immune response [12]. The *R* genes are the fundamental elements of genetic

interactions between biotrophic bacteria and plants which are known to control resistance against bacterial attack [13].

The majority of the sequences of cloned plant disease resistance genes, which provide resistance to the bacterial, viral, or fungal pathogens show that these consist of similar structural motifs and sequences [14]. The *Arabidopsis* genes, *RPM1* and *RPS2*, provide resistance against bacterial blight caused by pathogen *Pseudomonas syringae*; the rice gene *Xa21* confers resistance to *Xanthomonas oryzae*; the tomato gene *Cf-9* confers resistance to the fungal pathogen *Cladosporium fulvum*; the flax gene *L6* confers resistance to a rust fungus and the tobacco gene *N* confer resistance to tobacco mosaic virus; all these contain leucine-rich repeats (LRR) that encode protein motifs often linked with protein-protein interactions or ligand binding [15]. The resistance genes of *Arabidopsis thaliana* and *G. max*, *RPM1* and *Rpg1b*, respectively, mediate recognition of the same Type III effectors protein, AvrB, from *P. syringae*. *RPM1* and *Rpg1b* both belong to the coiled-coil nucleotide-binding sites (non-TIR or CC-NBS) class of resistance gene and they share only limited sequence similarity outside the conserved domains. Kanazin *et al.* [16] suggested that the similarities among resistance genes make it feasible to take advantage of sequence homologies for identification of other resistance genes. Resistance (*R*) genes cloning is the most recent method for obtaining disease resistant in legume crops [17].

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Genetic and physical localization of *G. max* (Williams 82) *Rpg1b* disease resistance gene showed a complex locus consisting of various tightly linked families of NBS-LRR gene. 1 Mega base (Mb) region of soybean cultivar Williams 82 centered on the *Rpg1b* disease resistance gene on chromosome 13, which is effective against certain races of bacterial blight. This region also contains numerous NBS-LRR type genes, out of which 52D1\_8 gene is also there, which belongs to non-TIR class like *Rpg1b* gene and hence it has similarity with *Rpg1b* gene of Williams 82. This is a putative disease resistance gene; whose sequence is devoid of introns [18-22]. The genome of Williams 82 has been completely sequenced [23]. However, very little is known about the genome of Indian soybean varieties at molecular level. Identification of resistance gene analogues in the Indian varieties is expected to aid significantly in the breeding programs of soybean. This study aims to amplify the disease resistance genes from the Indian varieties of *G. max* and to determine variations in sequences and functions of genes among these varieties.

## 2. MATERIALS AND METHODS

### 2.1. DNA Extraction from Selected Genotypes of Different Legumes

#### 2.1.1. Plant material/tissue

Seeds of 11 different varieties of soybean (NRC-37, JS-20-69, JS-20-98, NRC-86, JS-20-29, NRC-7, RVS-2001-18, NRC-121, JS-20-34, JS-97-52, and NRC-12) of soybean (*G. max*) were collected from the Indian Institute of Soybean Research, Indore (IISR). After 22–25 days of sowing, when seedling developed into healthy plants, young unopened leaves were plucked for DNA isolation.

#### 2.1.2. Reagents and chemicals

All the chemicals used in this study were of high-quality analytical grade reagents from HiMedia (MB502-HiPurA™ Plant DNA Isolation Kit, CTAB Method).

#### 2.1.3. DNA extraction

Genomic DNA was extracted from the young unopened/fresh leaves collected directly from the plants and brought over the ice from the pots to the laboratory. Total DNA was extracted using CTAB method as described by Doyle and Doyle [24] with some modifications.

#### 2.1.4. DNA purification

Purification of the extracted DNA was done to remove RNA, proteins, and polysaccharides, the major contaminants of the DNA precipitate. However, inclusion of CTAB in the DNA extraction buffer helps in the elimination of polysaccharides [25]. RNA was removed by RNase treatment and proteins were removed by phenol-chloroform extraction.

### 2.2. Polymerase Chain Reaction (PCR) Primers were designed from Selected Disease Resistance Genes

#### 2.2.1. Tools used

Different web-based resources/tools were used for primer design such as Primer3, Primer-BLAST, and ClustalW software.

#### 2.2.2. Primer design

The DNA sequences of disease resistant gene, *Rpg1b* (*G. max*), were retrieved from NCBI [26] and multiple sequence alignment (MSA) was performed using ClustalW [27]. After the alignment results, the conserved region was taken and both the primers (forward and reverse) were designed using online tool/software, namely, Primer3.

Set of primers (forward and reverse) for both NBS and LRR regions were constructed for disease resistance gene (*Rpg1b*) of *G. max*. The primers sequence in 5'–3' direction of both the regions of genes is shown in Table 1a and another primer set were used from bacterial artificial chromosomes (BAC) libraries of *Rpg1b* in Table 1b. These primers were selected from the NBS-LRR genes 21-F22\_29, 52-D1\_8, and 42i-18\_2 from soybean BAC 21-F22, 52-D1, and 42i-18, respectively. Standardization of NBS and LRR of *Rpg1b* for *G. max* and 5 more primer sets, labeled with A, B, C, D, and F, was done. PCR amplification was carried out according to the reaction mixture and conditions described below using these primers.

## 2.3. PCR Amplifications and Sequencing

### 2.3.1. PCR and purification of its amplified products

For PCR amplification, the reaction mixture was consisted of 2.5 µl of 10× polymerase buffer, 100 µM of each of the four dNTPs, 2.5 mM MgCl<sub>2</sub>, 25 ng of each of the forward and backward primers, 20 ng of genomic DNA, and 1.2 units of *Taq* DNA polymerase in a total of 25 µl volume. A master mix was prepared using PCR Recipe [28]. The genomic DNA was added separately after transferring master mix in PCR tubes. The reaction mixture after addition of all the components was mixed gently and PCR was carried in the thermal cycler (Amplifon make) using the thermal cycling conditions, as shown in Table 2.

**Table 1a:** Nucleotide sequences of selected primers.

Primers name		Primers sequence (5'–3')
Rpg1b_NBS	F	ATGCTGTGTTTGACGCAGAG
	R	TGCAATCTGGATTGTGTTGA
Rpg1b_LRR	F	CAAAAGCAACCCGTCATTTT
	R	ATCCCATCTCTCCATTCC

**Table 1b:** Second sets of standard primers with their sequence.

Label	Primers name	Primers sequence (5'–3')
A	21-F22_29	F1 ATGTTCTCAACAGGGTAAGAGACC
		R1 ACTTCCATGAAGATTGAGTTCTCC
B	21-F22_29	F2 ATGTTCTCAACAGGGTAAGAGACC
		R2 ATCCCATCTCTCACTTCACTTGTAC
C	52D1_8	F3 AATTCATCATGTAGTGTGGTGTCC
		R3 GAGAAACAAGAGAGTGTGGCAGTA
D	52D1_8	F4 CAAGCAACACAGAGAGAACAGATT
		R4 AAGTGTGTTGAAGGCTGTAGATTC
F	42i-18_2	F4 ACTCAATCTTCATGGAAGGCTATC
		R4 CTAATTGGGGGCACTCTATGATAC

**Table 2:** Thermal cycling conditions for polymerase chain reaction amplification.

S. No.	Step	Temperature (°C)	Time
1.	Initial denaturation	95	5 min
2.	Denaturation	95	30 s
	Annealing	60	30 s
	Elongation	72	60 s
3.	Final elongation	72	30 s

Repeat step 2 from Table 2 for 35 times at 4°C. PCR products were checked on 2.0% agarose gel along with appropriate size marker to check desired amplicon; PCR amplicon was purified by column purification to remove contaminants, present if any.

### 2.3.2. Gene sequencing

DNA Sanger sequencing method was used for sequencing of selected genes:

Rpg1b\_NBS – NRC-37 (S1), NRC-86 (S4), JS-20-29 (S5) and JS-20-34 (S9)

Rpg1b\_LRR – NRC-37 (S1), NRC-86 (S4), JS-20-29 (S5) and JS-20-34 (S9)

DNA sequencing reaction of PCR amplicon was carried out with same PCR primers (F and R) using BDT v3.1 Cycle sequencing kit on ABI 3730 × 1 Genetic Analyzer. Obtained sequences were analyzed for its quality in Applied Biosystems sequencing software at the genomics facility of GeneXplore, Ahmedabad. Thereafter, MSA, Boxshade of the DNA sequences was performed for confirming the presence of *Rpg1b* gene and to check the sequence variations among them.

**Table 3:** Varieties of different Indian soybean leaf samples.

S. No.	Varieties name	Leaf sample	Lane number
Soybean ( <i>Glycine max</i> ) varieties			
1.	NRC-37	S-1	1
2.	JS-20-69	S-2	2
3.	JS-20-98	S-3	3
4.	NRC-86	S-4	4
5.	JS-20-29	S-5	5
6.	NRC-7	S-6	6
7.	RVS-2001-18	S-7	7
8.	NRC-121	S-8	8
9.	JS-20-34	S-9	9
10.	JS-97-52	S-10	10
11.	NRC-12	S-13	11

**Table 4:** Primer optimization status.

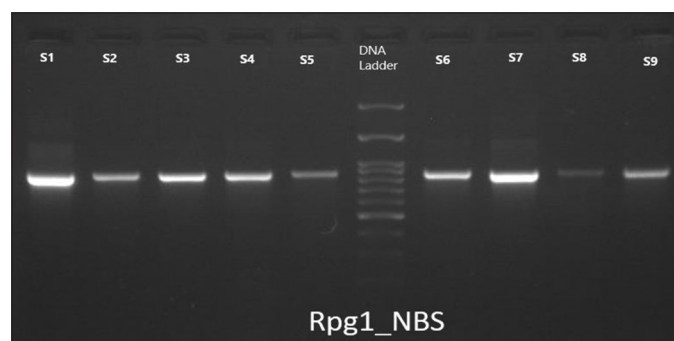
S. No.	Primer name	Amplicon status	Approx. band size (bp)	Annealing temperature (°C)
1.	Rpg1b_NBS	Amplicon obtained	900	55
2.	Rpg1b_LRR	Amplicon obtained	1000	55
3.	21-F22_29F1	No amplification	-	70
	21-F22_29R1			68
4.	21-F22_29F2	No amplification	-	70
	21-F22_29R2			68
5.	52D1_8F3	No amplification	-	68
	52D1_8R3			70
6.	52D1_8F4	Amplicon obtained	811	68
	52D1_8R4			68
7.	42i-18_2F4	Amplicon obtained	968	68
	42i-18_2R4			70

Total successful amplification obtained: 04/07

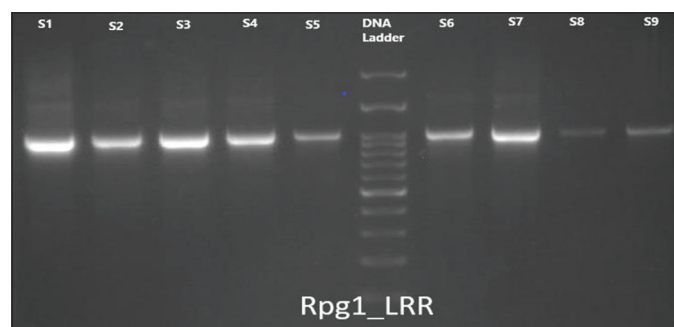
## 3. RESULTS AND DISCUSSION

### 3.1. DNA Isolation

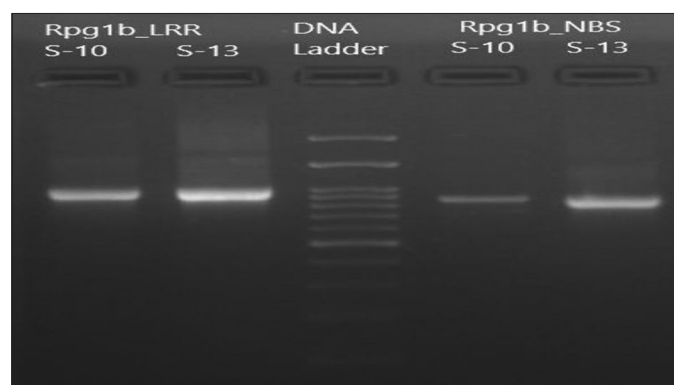
Genomic DNA from 11 different Indian cultivars of *G. max* was isolated by CTAB method, purified and amplified using Rpg1b\_NBS and Rpg1b\_LRR primers in PCR. Variety name of different Indian soybean leaf samples is mentioned in Table 3 and genomic DNA was extracted from sample S1 to S10 and S13 of soybean (*G. max*).



**Figure 1:** Gel description of samples S1 to S9 with primer Rpg1b\_NBS where well 1: NRC-37, well 2: JS-20-69, well 3: JS-20-98, well 4: NRC-86, well 5: JS-20-29, well 6: 100 bp ladder, well 7: NRC-7, well 8: RVS-2001-18, well 9: NRC-121, and well 10: JS-20-34 (Band approx. 900 bp).



**Figure 2:** Gel description of samples S1 to S9 with primer Rpg1b\_LRR where well 1: NRC-37, well 2: JS-20-69, well 3: JS-20-98, well 4: NRC-86, well 5: JS-20-29, well 6: 100 bp ladder, well 7: NRC-7, well 8: RVS-2001-18, well 9: NRC-121, and well 10: JS-20-34 (Band approx. 900 bp).



**Figure 3:** Gel description of the samples S10 (JS-97-52) and S13 (NRC-12) with primers Rpg1\_LRR and Rpg1\_NBS.



NM_001353616.1	241		GACGCAGAGGATATCTTGGATGAAATACACATGAGATCTC--CAATGCCAGCTCGAG
S1_NBS		1	-----CTCTTACACATGAGATCTC--CACATGCCAGCTCGAG
S4_NBS		1	-----CAGCGCCAAATGAGATCTC--CA-ATGCCAGCTCGAG
S5_NBS		9	TCTTCTGTAATTGCTCCAGGAGGT-TTCTTGTGCTGATGTTGAGCAGCTCTT
S9_NBS		1	-----CATGTCATCT--CA-ATGCCAGCTCGAG
NM_001353616.1	299		CTGAT--ECTGAAGC--TGAGTCT--CAACCTGCTCTTGCAAGGTACCAAAATTTCTTCAAA
S1_NBS		38	CTGATGCTCGAAGC--TGAGTCT--CAACCTGCTCTTGCGAGGTACCAAAATTTCTTCAAA
S4_NBS		38	CTGA--TCTGAAGC--TGAGTCT--GAAAGCTGTTCTTGGGAGGTACCAAAATTTCTTCGA
S5_NBS		69	CTACCTTCTGT--GCAAT--ATTCTACTCCCTGAGCCCAAAACAGATTTTATGACACA
S9_NBS		30	CTGA--ECTGAAGC--TGAGTCT--CAACCTGCTCTTGCAAGGTACCAAAATTTCTTCAAA
NM_001353616.1	355		TCTTCTCTGCTAGTTCCCTTTAACAGGGAAATTAATCCAGGATG--GAAGAAATCCTTGA
S1_NBS		95	TCTTCTCTGCTAGTTCCCTTTAACAGGGAAATTAATCCAGGATG--GAAGAAATCCTTGA
S4_NBS		92	TCTTCTCTGCTAGTTCCCTTTAACAGGGAAATTAATCCAGGATG--GAAGAAATCCTTGA
S5_NBS		129	GCTTCTCTGCTAGTTCCCTTTAACAGGGAAATTAATCCAGGATG--GAAGAAATCCTTGA
S9_NBS		85	TCTTCTCTGCTAGTTCCCTTTAACAGGGAAATTAATCCAGGATG--GAAGAAATCCTTGA
NM_001353616.1	414		TAGATTAGATCTTCTCTCAGC--CAAAAGGATGATCTAGGCTTGAAAAAT--TCTAGTG
S1_NBS		154	TAAATTAGATCTTCTCTCAGC--CAAAAGGATGATCTAGGCTTGAAAAAT--TCTAGTG
S4_NBS		151	TAAATTAGATCTTCTCTCAGC--CAAAAGGATGATCTAGGCTTGAAAAAT--TCTAGTG
S5_NBS		189	CCCTTCATTTTCTTCTCAGC--CAAAAGGATGATCTAGGCTTGAAAAAT--TCTAGTG
S9_NBS		144	TAAATTAGATCTTCTCTCAGC--CAAAAGGATGATCTAGGCTTGAAAAAT--TCTAGTG
NM_001353616.1	470		GTGTTGGCTTGGG--TCAGAATTGGCTATGTCAGTACCACATATACCAATCAGATCT
S1_NBS		210	GTGTTGGCTTGGG--TCAGAATTGGCTATGTCAGTACCACATATACCAATCAGATCT
S4_NBS		207	GTGTTGGCTTGGG--TCAGAATTGGCTATGTCAGTACCACATATACCAATCAGATCT
S5_NBS		249	GATTTTCTATGGCTTCAATGATTTCTTGTGTTCCCTTAAACATCAGATCT--ATCT
S9_NBS		200	GTGTTGGCTTGGG--TCAGAATTGGCTATGTCAGTACCACATATACCAATCAGATCT
NM_001353616.1	529		TCAAGTTGTTGAAAGTATTTTATGGCAA--GATAAAGC--AAAAAATGATTTTGTAT
S1_NBS		269	GTTTGTGTTGAAAGTATTTTATGGCAA--GATAAAGC--AAAAAATGATTTTGTAT
S4_NBS		266	TCTGTTGTTGAAAGTATTTTATGGCAA--GATAAAT--AAAAAATGATTTTGTAT
S5_NBS		306	GAAAC--CAACCCAGCTCTGATCTCAATCTACCTCTCTATCTGCTGATTTGATGAA
S9_NBS		259	CGAGTGTGAAAGTATTTTATGGCAA--GATAAAGC--AAAAAATGATTTTGTAT
NM_001353616.1	586		TGCTGACCTGTGACAAATGCAATGCTAACCA--GCA--TGATACCTTCTATTTGTGGG
S1_NBS		326	TGCTGACCTGTGACAAATGCAATGCTAACCA--GCA--TGATACCTTCTATTTGTGGG
S4_NBS		323	TGCTGACCTGTGACAAATGCAATGCTAACCA--GCA--TGATACCTTCTATTTGTGGG
S5_NBS		365	TGC--AAATTTGACAAATGCTGCTTACCAATGCT--ACGCT--CCCTCAATGAAATG
S9_NBS		319	GCGCTGACCTGTGACAAATGCAATGCTAACCA--GCA--TGATACCTTCTATTTGTGGG
NM_001353616.1	643		ATGGGTGGGATGGGTAAACCACTTGTCTCAACATGTTTCATGACCCCTA--GGAT
S1_NBS		383	ATGGGTGGGATGGGTAAACCACTTGTCTCAACATGTTTCATGACCCCTA--GGAT
S4_NBS		380	ATGGGTGGGATGGGTAAACCACTTGTCTCAACATGTTTCATGACCCCTA--GGAT
S5_NBS		420	ATGCTGGC--TGGTA--ATTGCGCTTCTCA--TGTCAACCAATGAA--ATCT
S9_NBS		379	CAAGGTGGGATGGGTAAACCACTTGTCTCAACATGTTTCATGACCCCTA--GGAT
NM_001353616.1	699		CHAGGAGGCTAGATTGTTGTCAAAGCTT--GGTCTGTGTTTCA--GATGATTT--TGAT
S1_NBS		439	THAGGAGGCTAGATTGTTGTCAAAGCTT--GGTCTGTGTTTCA--GATGATTT--TGAT
S4_NBS		436	THAGGAGGCTAGATTGTTGTCAAAGCTT--GGTCTGTGTTTCA--GATGATTT--TGAT
S5_NBS		473	TTTTTTTCTTCAATCT--TGCAATATTCCTCTCAACCACTGAGAGAT--TGATTT
S9_NBS		439	CHAGGAGGCTAGATTGTTGTCAAAGCTT--GGTCTGTGTTTCA--GATGATTT--TGAT
NM_001353616.1	754		GCTTTC--GG--CTTCA--GAAC--AATCTTGAGGCCATCACTAAAT--GACTGA--TGAT
S1_NBS		495	GCTTTCGGG--GG--GGGGAACCAATCTTTC--GGGCTCACTAAATCGCTGA--ATAT
S4_NBS		491	GCTTTCGGG--GG--GGGGAAC--AATCTTGAGGCCATCACTAAATCACTGA--TGAT
S5_NBS		531	GATATGCTG--GTCTGCTCACTACCAATCT--GATTCATG--CAACA--CCACT--GAT
S9_NBS		499	GCTTTCAGGATGCTCACTACCAATCTGCTGAGGCCATCACTAAATCACTGAATGAT
NM_001353616.1	808		--AGTAAGACCTAGAGATGGTTCAGG--GAAGATTGAAAGAAAAATG--CGGGAA
S1_NBS		552	--AGTAAGACCTAGAGATGGTTCAGCT--GAACATTAAAGAAAAATGCTGTTTAA
S4_NBS		547	--ATTAAAGACCTAGAGATGGTTCAGG--GAAGATTGAAAGAAAAATGCTGCGAA
S5_NBS		586	--TTTTCAAGCCTA--GATCTGCTTTT--TGATTGAAGAAATTCATCTTCAAG
S9_NBS		559	CGATTAAGACCACTAGAGATGGTTCAGG--GAAGATTGAAAGAAAAATGCAAGGAA
NM_001353616.1	861		GAAT--TTTCTCTG--TTTGGATGAC--TTTGA--CAAAAC--GACTTAAATG
S1_NBS		607	GATGA--TTTCTCTG--TTTGGATGAC--TTTGA--CAAAAC--GACTTAAATG
S4_NBS		603	AGATT--TTTCTCTG--ATTGGATGAC--TTTGA--CAAAAC--GACTTAAATG
S5_NBS		638	GATTTC--TTCTCTCTG--AGTAATTTCTCTGTTAAAGGAAGTACGAGAGAAATTT
S9_NBS		619	GTTTTCATGTTCTTCTAGGATGCGGATGAC--TTGAAACAAAAATGACTTAAATG
NM_001353616.1	913		--GAAC--TTTGCTAA--ACATCTTGGTTTGGCTCAG--GGTAG--TATTATGCCAG
S1_NBS		660	CTTATCTGTTGCTAA--ACATCTTGGTTTGGCTCAG--GGTAG--TATTATGCCAG
S4_NBS		655	CAAACT--TTTGCTAA--ACATCTTGGTTTGGCTCAG--GGTAG--TATTATGCCAG
S5_NBS		695	GAAGAAATTTGCTCTCAAGGCTGTTTGGCT--CACTCTCACTTTCAGGTTG
S9_NBS		679	GAACCTTCTGCTAAATACATCTTCTGATTTGGCTTCTCAAGTAAGGATTTATC
NM_001353616.1	969		CACACGTAGTAA--GAGTTGCTTCTACCA--GAGGTCA--GAC--CTCCTGGAGCAATT
S1_NBS			-----
S4_NBS			-----
S5_NBS			-----
S9_NBS		752	CAAGCTGGCATTTGAGATCTCAAGTTGTAATTTCACTCAAGATTTCTCTC
			CAACACTCAATTAAGGCTTTTGTGCTTTTCTCAATTTCACTTCTTAGAGCA--TT

Figure 4a: Multiple sequence alignment using ClustalW and Boxshade of NBS region of four Indian soybean varieties and Williams 82 (NM\_001353616.1).

Here, S1 is NRC-37, S4 is NRC-86, S5 is JS-20-29, and S9 is JS-20-34.

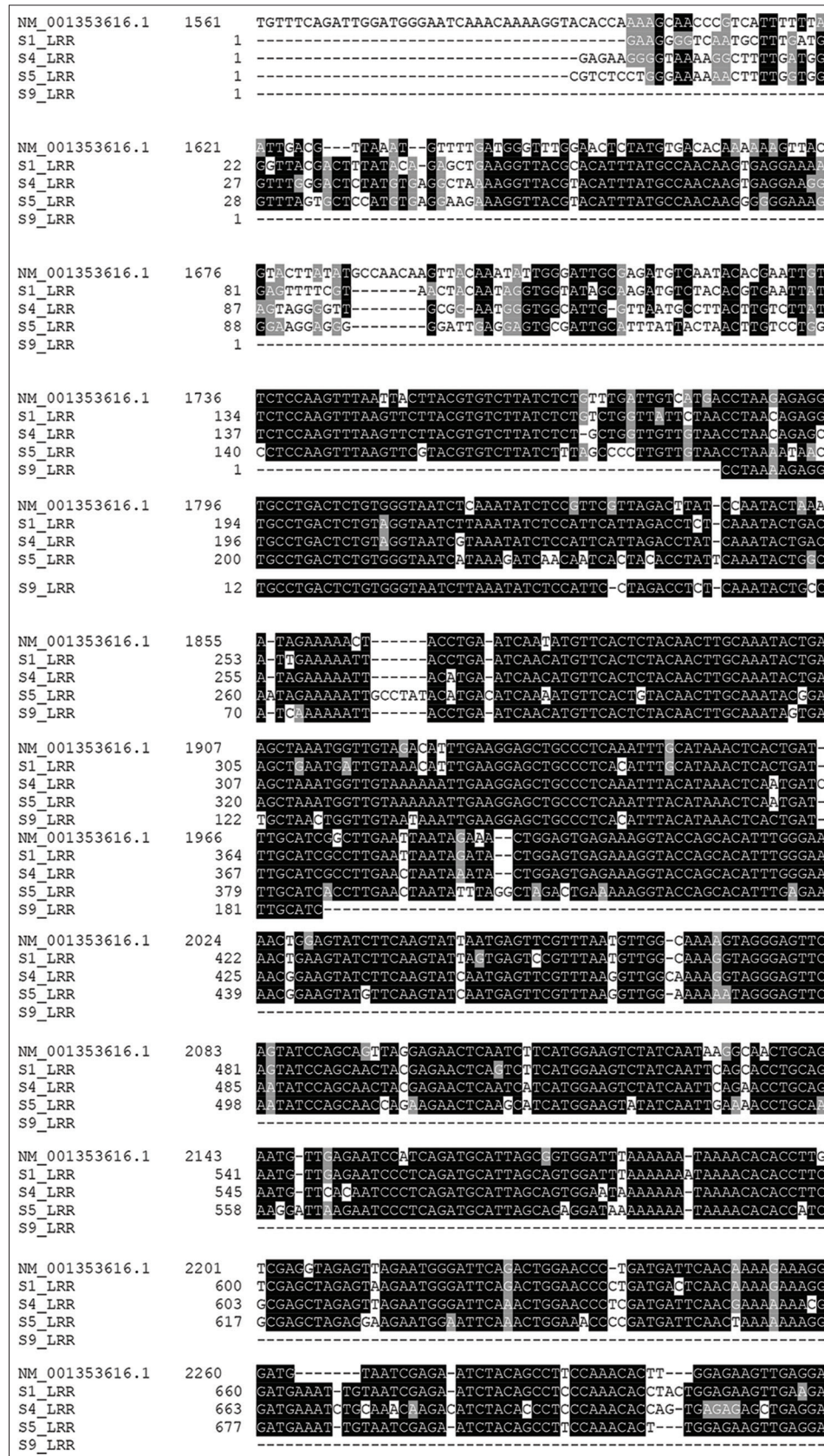


Figure 4b: Multiple sequence alignment using ClustalW and Boxshade of LRR region of four Indian soybean varieties and Williams 82 (NM\_001353616.1).

Here, S1 is NRC-37, S4 is NRC-86, S5 is JS-20-29 and S9 is JS-20-34.



### 3.2. PCR Optimization

Optimizing PCR amplifications, the results obtained are described in Table 4, where primers of *Rpg1b* NBS and LRR are showing amplification at annealing temperature 55°C and the approx. band size obtained is up to 900 bp. In primer sets, Rpg1b\_NBS and Rpg1b\_LRR amplification were obtained at all three annealing temperature used 52, 55, and 58°C. The primer optimization results as shown in Figures 1-3 for Rpg1b\_NBS and Rpg1b\_LRR, at 55°C temperature revealed clear and sharp band so it has been considered (55°C) for all remaining samples, that is, S1 to S13 of *G. max* except S11 and S12 as there is no DNA obtained.

### 3.3. Gene Sequencing

Out of 11 different varieties, four local varieties which are of economic importance and good yield were selected for further sequencing. DNA of ~1200 base pairs was successfully amplified from the four sample of *G. max* and sequenced. Sequence was closely matching with Williams 82 cultivar. These sequences were submitted in NCBI with GenBank accession no. MH700732.1 to MH700739.1.

Four Indian soybean varieties sequence were aligned using MSA tool with reference to *Rpg1b* gene (disease resistance gene consisting NBS-LRR regions) from Williams 82. Variations exist in Williams 82 region and Indian varieties, as shown in Figure 4a and b. In NBS region, variety S5 (JS-20-29) revealed maximum variations, while varieties S1 (NRC-37) and S4 (NRC-86) show the lowest variations. In LRR region, variety S9 (JS-20-34) has shown maximum significant variation and the variety S4 (NRC-86) shows minimum variations.

The previous studies have reported the amplification of disease resistance genes using PCR among numerous plant varieties [16,17,29]. In this study, using the primers for the NBS and LRR region of *Rpg1b* gene of Williams 82 soybean variety, we have amplified the NBS and LRR sequences from different Indian varieties of *G. max*. Yu *et al.* [30] reported the amplification and cloning of NBS sequences from soybean using degenerate primers of *N* and *RPS2* genes of tobacco and *Arabidopsis*, respectively. Chang *et al.* [31] have also reported the characterization of disease resistance loci in the USDA soybean germplasm collection using genome-wide investigations.

Earlier analyses have reported the successful use of primers corresponding to conserved NBS domains in *R* genes to amplify sequences from soybean [32]. The NBS regions were taken as they are conserved in all resistance genes of legume and LRR were considered, as they are responsible for pathogen recognition. Various forward and reverse primers were designed from the coding region of disease resistance gene (*Rpg1b*) sequences of *G. max* using primer3 tool. Using the set theories, permutation, and combinations, different sets of primers were selected. Furthermore, some universal primers of soybean were taken from different BAC libraries and amplified for confirming the amplification of PCR protocol.

Standardization of primers from Williams 82 on different Indian soybean varieties was done, which showed that all these genes (NBS-LRR) were present in Indian soybean cultivars. Both primers show amplification and found to be conserved among those cultivars. After sequencing of Indian varieties of *G. max*, we have compared the sequence with its analog in Williams 82 and concluded about sequence similarities and variations as shown in MSA, Boxshade results [Figure 4a and b]. The similarities in the sequence of these varieties with Williams 82 showed that disease resistance gene (*Rpg1b*) is present in these varieties. These variations among the different soybean cultivars revealed the genetic diversity in the disease resistance genes. Development of molecular

markers linked to these genes in various legumes has been done recently [33]. Molecular detection and characterization of resistance genes for bacterial blight will serve as an important resource for studying evolution of these genes from the legumes grown in Indian subcontinent. The practical use of this study is development of disease resistance in legumes, as well as in generating basic and fundamental knowledge on the resistance genes. Molecular characterization of these genes would be helpful in genetic modification to produce durable and disease resistance soybean varieties.

### 4. CONCLUSION

Presence of *Rpg1b* gene in four Indian soybean cultivars, that is, NRC-37 (S1), NRC-86 (S4), JS-20-29 (S5), and JS-20-34 (S9) was confirmed. Primers were used based on conserved NBS domain and LRR domain. Since *Rpg1b* is a disease resistance gene, which protects against bacterial blight, therefore, this gene sequences from selected Indian soybean cultivars will be used for developing *R* gene-based markers and for screening of bacterial blight in soybean. Apart from this, it will greatly facilitate the molecular cloning of disease resistance genes in soybean.

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### 6. CONFLICTS OF INTEREST

Author declared that he does not have any conflicts of interest.

### 7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

### 8. ETHICAL APPROVALS

Not applicable.

### 9. PUBLISHER'S NOTE

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