

Identification and Characterization of Disease Resistance Pathway Genes in Jute (*Corchorus spp.*)

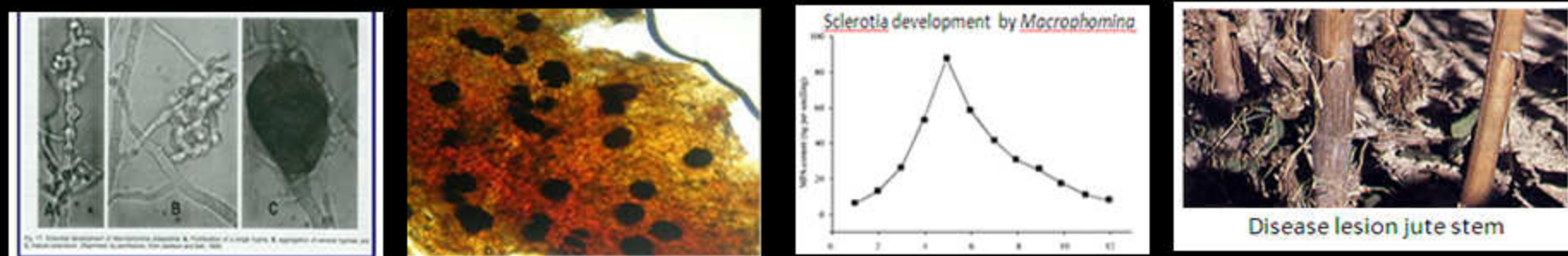
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Plant disease resistance genes, mainly known as the R genes, play a vital role in plant defense system through encoding proteins for pathogen detection. Most plant R genes have a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region. The NBS region is preceded either by a coiled-coil (CC) domain or a TIR (Toll/interleukin-1 receptor like) domain. With a view to identifying and characterizing all NBS domain containing genes in jute, degenerate primers were designed from the conserved NBS domain. Subsequent cloning and sequencing identified the presence of both CC-NBS-LRR and TIR-NBS-LRR domain containing disease resistance genes. Among all the NBS genes, RIN4, RPM1, RPS2 and RPP5 were

selectively targeted for expression analysis between the traditionally cultivated species *Corchorus capsularis* susceptible to disease, and the wild type resistant species *Corchorus trilocularis*. RPM1, RPS2 and RIN4 belong to plant innate disease resistance pathway whereas RPP5 works in systemic acquired resistance (SAR) pathway resulting in salicylic acid accumulation. Under fungal stress, the expression of these resistance genes increased significantly in wild type resistant species than the susceptible ones. This outcome allows us to hypothesize that *Corchorus trilocularis* possess competent disease resistance transcripts providing the opportunity to develop a transgenic disease resistant jute variety.

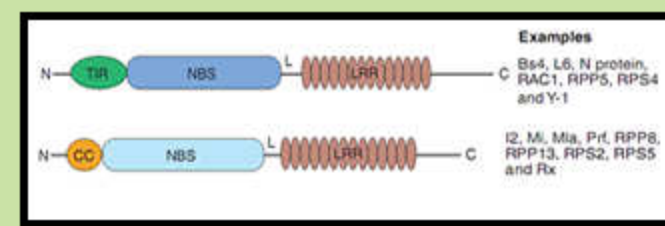
Introduction

Jute is the major fiber producing plant of our country. For having shiny golden fiber it is called the 'Golden Fiber' of Bangladesh. For our country jute is not just a plant that produces fiber; it is rather a national icon, linked to the adage- 'Sonar Bangla'. However, every year the production of jute is drastically hampered due to different types of pathogen infection. Among these the fungi *Macrophomina phaseolina* causes the devastating Root-knot disease.



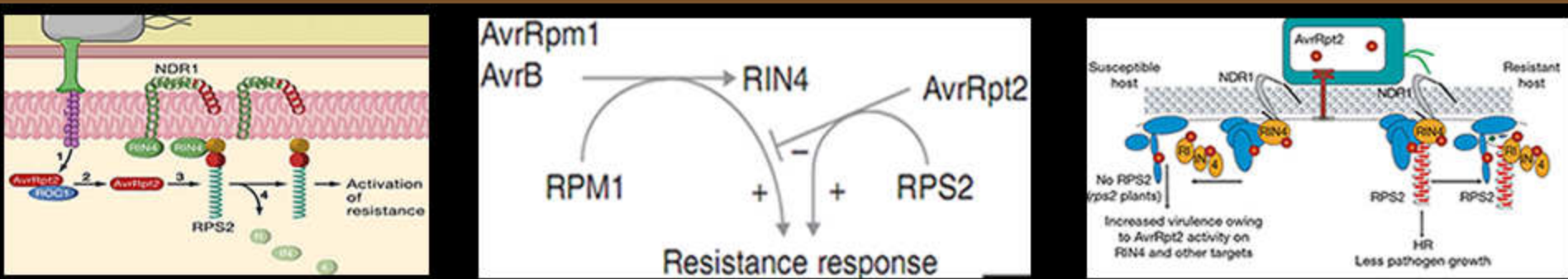
Macrophomina may cause infection and damage at all stages of growth of the plants, right from seedling emergence to maturation

Nonetheless, plants have adapted some resistance pathway against the diseases to some extent. Majority of disease resistance genes (R genes) in plant contain NBS-LRR



domain. This large protein family subdivided into two distinct classes: TIR-NBS and CC-NBS. NBS domain is common in both cases which is conserved but TIR and CC domain varies according to the type of pathogens. Plant NBS-LRR domain shows similarity with mammalian Nucleotide Binding Oligomerization Domain (NOD-LRR protein) which function in inflammatory response. Plant R gene confers several resistant mechanisms against pathogens. Guard hypothesis is the most vital one covered by RPM1, RIN4 and RPS2 proteins. Another important disease resistance pathway is Systemic Acquired Resistance (SAR) which is a little bit adaptive pathway in plant to defeat pathogens.

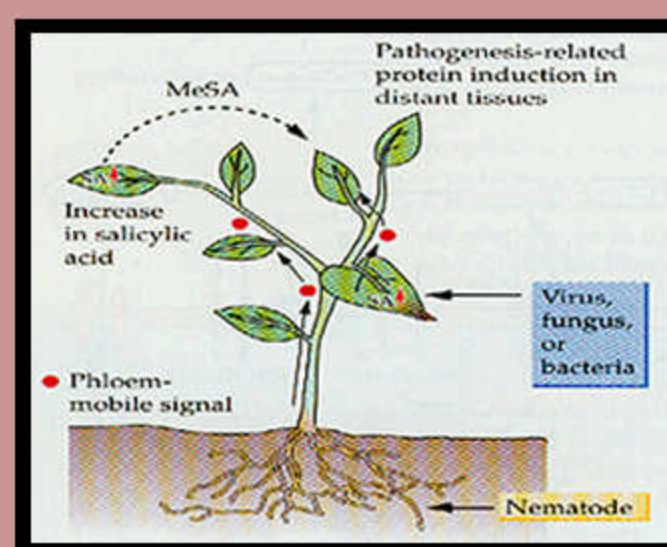
Mechanism of Guard Hypothesis



- Pathogens protein Avr phosphorylates plant R protein RIN4. RPM1 detects the phosphorylation of RIN4 and elicit the resistance response.
- Avrpt2 from pathogen abrogate this response. This inhibitory effect is overcome by RPS2 and resistance is acquired.

Systemic Acquired Resistance (SAR)

- SAR is a generalized resistance pathway after exposure to pathogen. It is a mobile infection induced defense system. At first localization of effector molecules such as salicylic acid, jasmonate occurs which then spread systemically throughout the plant and provide resistance against further infection.



- These molecules actually activate a transcription factor namely NPR1. This NPR1 transcription factor further activates some downstream pathogenesis related (PR) proteins. Further spreading of pathogens is then inhibited through local lesion, necrosis, and cell death.

Aim of the study

- Identification and characterization of all NBS containing genes in jute using degenerate primers designed from the conserved NBS domain.
- Corchorus capsularis* and *Corchorus trilocularis*- two jute species were used for this purpose as these are susceptible and wild type resistance variety respectively.
- Evaluation of two different disease resistant pathway through four degenerate primers for the RPP5, RPM1, RPS2 and RIN4 genes.
- Expression analysis of these genes under different biotic stress conditions.
- Bioinformatics analysis of these genes and building a network of the genes in Arabidopsis

Methods

- Designing of degenerate primers from the conserved domain of the genes.
- Confirmation of the desired genes in jute genome through PCR.
- Sequencing of the amplified products.
- Gene specific primer design.
- Biotic stress mainly fungal stress will be conferred using *Macrophomina phaseolina*.
- Comparative expression analysis.
- Establishment of a network of these proteins by using STRING9.0 database (<http://string-db.org/>).

Discussion

- We got band using NBS-2 primer (Fig-2) and after sequencing blast result shows similarity with TRAF proteins of Arabidopsis which is a member of interleukin-1 receptor/Toll family. This family of receptors is involved in host defenses against microbial pathogens.
- Positive result was obtained by RPM1 (Fig-4) and RPS2 (Fig-5). Sequence of RPM1 gene shows similarity with Brassica rapa RPM1 disease resistant gene and RPS2 shows similarity with Vitis vinifera pathogen resistance gene. As these two genes are present in jute, it is suspected that other genes of the pathway will also present.
- Network of Guard hypothesis pathway genes indicates that, beside RPM1, RPS2 and RIN4 there are other four interacting partner which are important for providing resistance against pathogens. Similarly, RPP4, a homologue of RPP5 protein, forms a cluster with some other genes that demonstrates the potential disease resistance pathway in jute.

References

- Leah et al, Plant NBS-LRR proteins: adaptable guards, *Genome Biology*, doi: 10.1186/gb-2006-7-4212
- Daniel F. Klessing et al, Nitric oxide and salicylic acid signaling in plant defense, *Colloquium*

Result

- Presence of NBS containing genes in the genomes of both jute species was confirmed.
- We used three pairs of NBS primer, among these we got bands for NBS-2 and NBS-4 by PCR.
- Sequencing of these gene was performed.
- In *Corchorus trilocularis*, a similarity was found with TRAF disease resistant gene using NBS-2 primer.

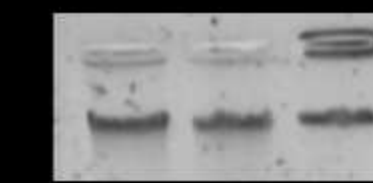


Fig. 1: Genomic DNA isolated from two jute species. Lane 1: *Corchorus capsularis*, Lane 2: *Corchorus trilocularis*, Lane 3: Lambda DNA.

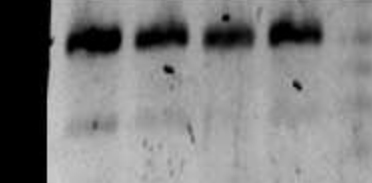


Fig. 2: PCR with NBS-2. Lane 1: *Corchorus capsularis*, Lane 2: *Corchorus trilocularis*, Lane 3: *Corchorus trilocularis*, Lane 4: 1 kb+ ladder



Fig. 3: PCR with NBS-4. Lane 1: *Corchorus capsularis*, Lane 2: *Corchorus trilocularis*, Lane 3: 1 kb+ ladder

Fig. 4: PCR with RPM1. Lane 1: *Corchorus capsularis*, Lane 2: *Corchorus trilocularis*, Lane 4: 1 kb+ ladder

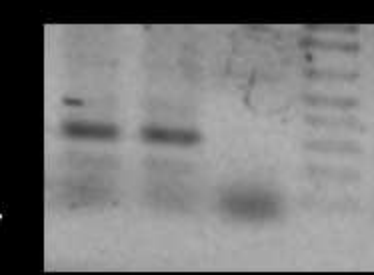
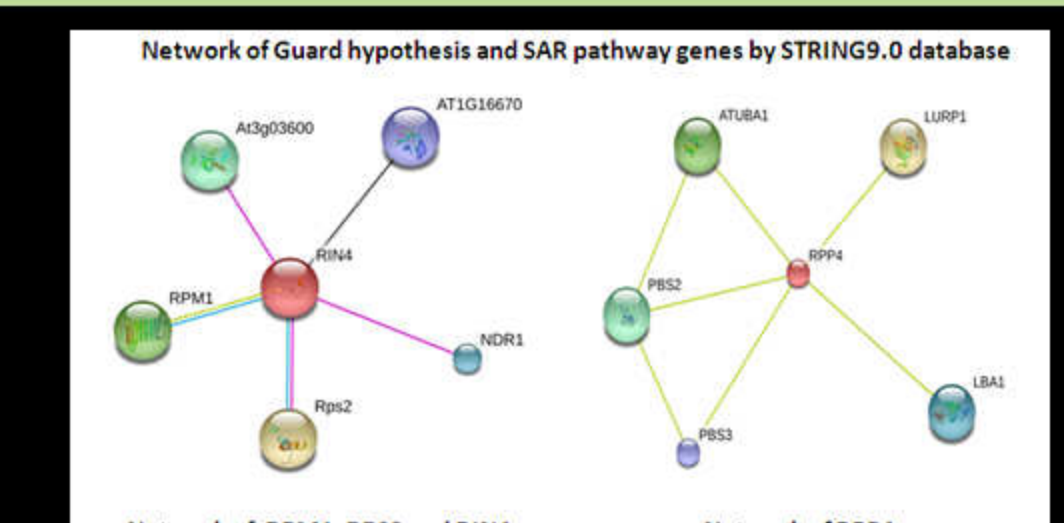


Fig. 5: PCR with RPS2. Lane 1: *Corchorus capsularis*, Lane 2: *Corchorus trilocularis*, Lane 3: 1 kb+ ladder

- Blast result, using the sequences, showed significant similarity with disease resistant genes in case of RPS2 and RPM1.

A					
Accession	Gene	Length	Score	E-value	Identity
U00001	Arabidopsis thaliana TRAF1 protein (U00001) - contig	221	314	1.7e-11	88%
U00002	Arabidopsis thaliana TRAF1 protein (U00002) - contig	221	314	1.7e-11	88%
U00003	Arabidopsis thaliana TRAF1 protein (U00003) - contig	221	314	1.7e-11	88%
U00004	Arabidopsis thaliana TRAF1 protein (U00004) - contig	221	314	1.7e-11	88%
U00005	Arabidopsis thaliana TRAF1 protein (U00005) - contig	221	314	1.7e-11	88%



- Presence of RPP5, RPM1, RIN4, RPS2 genes have also been confirmed in jute genome by PCR.

What Next

- M. phaseolina*, *P. parasitica* and *P. syringae* will be used to confer biotic stress.
- Temporal expression of RPM1, RPS2, RIN4 and RPP5 will be observed through semi quantitative PCR.
- Comparative analysis of two different resistance pathway in both jute species.
- As *C. trilocularis* is a wild type resistant variety so it is expected that the resistance pathway here will be different from *C. capsularis*. This information will be used to develop biotic stress resistant jute variety.