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Characterization and Molecular Analysis of NBS-LRR Class Resistance Gene Homologues Isolated from *Zingiber* spp. of North East India

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Abstract: ***Rhizome rot or Fusarium yellowson ginger is a serious soil-borne disease of gingerin areas it is grown and found to be prevalent in the Northeastern region of India as well. In this region, it is mainly caused by the fungus Fusarium oxysporum f. sp. Zingiberi. Most of the plant disease resistance (R) genes encode a highly conserved nucleotide binding site and leucine-rich repeat structure (NBS-LRR). Therefore, degenerate primers based on the conserved domains ofresistance genes were obtained to target PCR to amplify resistance gene candidates (RGCs) from Zingiber spp.OfNortheast India. PCR amplification from genomic DNA yielded a group of fragments of approximately 350bp and 600bp DNA sequences. Cloning, sequencing and characterizationof the sequences showed thattheamino acid sequences of the RGCs,detected the presence of conserved motifs of superfamilies NS-ARC and significant homology with R-genes in the GenBank database from other plant species. The level of sequence identity between Zingiber RGC sequences and known resistance genes in the top blast hits varied from 87% to 91% between Zne19p6 and Zne31p6 respectively to Zingiber officinale clone ZoP26 (e-value: 1e-106) and Zingiber zerumbet clone ZzP226 (e-value: 1e-81). RGCs were also detected using the RGC-specific primers designed. However, in RT-PCR analysis, the expression of the disease resistance seems to be very low or absent in the Zingiber species found in the region. The RGCs can be used to characterize resistance genein related plant species to further study the organisation and functioning of such NBS-LRR encoding R-genes in asexually reproducing plant species. This study reports for the first time on characterization and molecular analysis of FusariumRGCsfrom Zingiber spp. of North east India.***

Keywords: Degenerate primers, R-genes, Resistance gene candidates (RGC), NBS-LRR class, *Fusarium oxysporumf.* sp. *Zingiberi*

1. Introduction

Ginger (*Zingiber officinale Rosc*., family Zingiberaceae 2n=22), is an important commercial crop in tropical and subtropical countries. Ginger is asexually propagated from portions of the rhizome. Globally India is the largest producer and exporter of the finest quality ginger. Ginger is used throughout the world as a spice or fresh herb or in medicines. Cultivated ginger originated in India or Southeast Asia [1]. Globally, the main producers of ginger are India, China, Nepal, Indonesia and Nigeria [2].India is the largest producer and exporter of the finest quality ginger. India's production of ginger constitutes about 34.6 % of the total world's production of ginger[3].Ginger is grown in almost all the states of the North-Eastern region of India.Assam ranked first in ginger acreage as well as in production but productivity was highest in Mizoram, followed by Arunachal Pradesh, Assam and Nagaland[4]. A number of local cultivars of ginger are also found in North-Eastern region. These varieties are high yielder of rhizomes as compared to standard cultivars, but have more fiber content.

Ginger is one of the most promising spice crop grown in North Eastern India. It is estimated that more than 50% of the national production of ginger comes from the North Eastern States. The soil and climate of the region, enormously favour the growth of the cropand as such there is a tremendous scope to develop for an increasein its yield per unit area.But the continuous domestication of preferred genotypes of ginger and their exclusive vegetative propagation has resulted in the degradation of the genetic base of this crop. Due to this almost all the cultivars available today are equally susceptible to all major diseases.

In India, rhizome rot and yellows caused by *Fusarium oxysporum* f. sp. *Zingiberi* (fusarium yellows on ginger) is a big threat to the production of this crop [5]. Rhizome rot is found to be prevalent in many areas of North east India as well. As ginger is an obligatory asexual crop, resistance breedingislimited only to itsgermplasm screening [6]. Till now no work has been taken up to evaluate the wild relatives of ginger for *Fusarium* wilt resistance in North east India. Therefore, the genetic resource of ginger needs to be accessed for identification of *Fusarium* resistance. As such,the most sought after techniques of genetic improvement for disease management, could be applied to increase the yields of the crop in the region. Resistance gene candidates (RGCs) could be utilized to investigate features of resistance-related loci in ginger for its genetic improvement.

Plant disease resistance genes (R-genes) constitutean important component of the genetic resistance mechanism in plants [7] [8]. R-genes seem to have a significant role in recognizing proteins expressed by specific avirulence (Avr) genes of pathogens [9].The NBS-LRR *R*-genes are found to be abundant in plant genomes with approximately 150 and 600 isolated from *Arabidopsis* and rice respectively [10] [11].

About 75% of plant *R*-genes encode proteins with a nucleotide-binding site and leucine-rich repeat (NBS-LRR) domain that provide resistance to various pests and pathogens such as bacteria, fungi, viruses, insects and nematodes [8]. It has been reported that the C-terminal LRR acts as a site for pathogen recognition and the N-terminal

NBS initiate signaling which activates signal transduction pathways leading to disease resistance in the plant [12].

Wild relatives of many other plants have been reported to be used as an important source of genetic variation for disease resistance [13] [14]. In this context, molecular characterization of resistance-related sequences from ginger and its wild relatives may help us retrievedisease resistance specificities for the improvement of ginger.

2. Literature Survey

Plant resistance genes (R-genes) are reported to play a key role in plant defence and form a vital component of the genetic resistance mechanism in plants [7] [8]. They seem to play a significant role in recognizing proteins expressed by specific avirulence (Avr) genes of pathogens [9]. It has been recently shown that proteins encoded by resistance genes display modular domain structures and require several dynamic interactions between specific domains to perform their function. Some of these domains are for proper interaction with Avr proteins and in forming signalling complexes that will activate an innate immune response to arrest the proliferation of the invading pathogen. R-genes have been cloned from a wide range of plant species either by map-based cloning [15] [16] or transposon tagging [17] [18] from model systems or species with a long history of genetic research.

The cloning of genes for resistance against diverse pathogens from a variety of plants in earlier works has revealed that many share conserved sequence motifs. This gives us the possibility of further isolating numerous additional resistance genes by polymerase chain reaction (PCR) with degenerate oligonucleotide primers.It has been successfully employed for the isolation of homologous sequences called resistance gene candidates (RGCs) from many plant species[19] [20] [21] [22] [23] [24] [25] in different plant species such as soybean [20] [26], maize [27], lettuce [23], rice [28], common bean [24], citrus [29], wheat [30], sorghum [31], and ginger [32]. The RGC fragments has been used as molecular markers for tagging the disease resistance loci in *Arabidopsis* [21], rice [33], tomato [34], etc. RGC sequences amplified following candidate gene approach has providedmajor information about the organization, distribution and evolution of R-genes/RGCs [20] [35] [36] [10] [37]. The RGCs have become a promising tool for the isolation of full-length resistance genes from crop plants such as wheat [38], common bean [39], soybean [26] and citrus [40].

Moreover, the structural analyses of cloned resistance genes have led to the identification of conserved regions in the structural domains and sequences involved in protein-protein interaction and signal transduction [41] [42]. Based on these conserved motifs, R-genes have been grouped into five classes [43].

Class I is represented by the maize *HM1* gene, encoding a reductase that detoxifies HC toxin of the fungus *Cochliobolous carbonum*. Class II is represented by the majority of functionally known R-genes (*RPS2*, *RPM1*, *N*, *L6*, etc.), which encode cytoplasmic receptor-like proteins that contain a leucine-rich repeat (LRR) domain and nucleotide-binding site (NBS). Class III includes the *Pto* gene from tomato, which does not have an NBS-LRR domain, but encodes a protein with a serine-threonine protein kinase domain. Class IV includes the *Xa21* gene of rice, which encodes an extra-cytoplasmic LRR domain and an intracellular serine/threonine kinase domain, while class V represents the *Cf* genes of tomato that encode transmembrane receptors with an extracellular LRR domain and an intracellular serine-threonine kinase. It has been observed that most of the known R-genes possess conserved amino acid motifs along the NBS sequence. This includes the P-loop and the kinase-2 domains, which are ATP- and GTP-binding sites [44] [45], the kinase-3a domain and the putative membrane spanning hydrophobic GLPL domain [46].

Wild relatives of many other plants have been used as an important source of genetic variation for disease resistance [13] [14] since they can evolve resistance specificities more efficiently than cultigen [47] [48]. In this context, molecular characterization of resistance-related sequences from ginger and its wild relatives may provide a lead towards retrieving resistance specificities suitable for the improvement of ginger.

3. Materials and Methods

3.1 Plant Materials collected

Species of genus *Zingiber*, *Z. montanum*, *Z. zerumbet*var.darcyi, *Z.officinale* Roscoe var. Nadia, *Z.officinale* Roscoe var. Baishy, *Z.officinale* Roscoe var. Meitei shing, *Z. zerumbet(L.)* Smith, *Z. kerrii* Craib, *Z. rubens* Roxb, *Z. sp3*, *Z. sp1*, *Z.sp2* were obtained from Bioresource Park, Institute of Bioresources and Sustainable Development (IBSD),Hararou, Manipur, Indiawhere the cultivars are maintained as accessions under shade house conditions. Apart from these, *Zingiber* spp. spreading across different locations of North east were collectedfrom farmer's fields and maintained as accessions in greenhouse which include, *Z. purpureum*, *Z. roseum*, *Z. zerumbet* (L.) Smith, different cultivars of *Z. officinale*, *Z. montanum* and Meitei shing.

3.2 Isolation of DNA

Total genomicDNA was extracted from young leaves using CTAB method using the procedureof Doyle and Doyle (1990) [49] with minor modifications. DNA was diluted to 20 ng/µl final concentration in sterile deionised water and storedin 1X TE buffer (10 mM Tris Cl pH 8, 1 mM EDTA pH 8) at -20 °C.The quantity and quality ofDNA preparation were verified by standard spectrophotometry methods (NanodropSpectrophotometer ND 2000) and visualized on 0.8% agarose gel stained withethidium bromide.

3.3 Primers and PCR amplification

A total of 10Resistance gene specific degenerate primers (Table 1, Sigma Aldrich ChemicalsPvt. Ltd., India) previously used in published literature foramplifying RGCs in other cropswere selected. PCR reaction was carried out in

a volume of 25 µl containing 1 unit Taq DNA polymerase, 10X PCR buffer, 1.5 mM of MgCl2, 200 mM of dNTPs, 20 picomole of each primer and 30 ng of template DNA, . PCR amplification was carried out in a thermal cycler Eppendorf Mastercycler pro Sprogrammed for an initial denaturation at 94°Cfor 5 min, followed by 35 amplification cycles, 94°C for 1 min, 55°C for 1 min and 72°C for 1 min and a finalextension step at 72°C for 5 min.

Table 1: List of RGC specific degenerate primers used for the PCR amplification of Zingiber resistance gene candidate

Primer	Sequence (5' → 3')		Targeted domain
	Forward	Reverse	
FR1	TGGTGG GGTTGGGAA GACAACG	TCCCGCTAGTGGCAA TCCCTAG	NBS-LRR/P-loop:NBS-LRR
FR2	GGIGGIGTIGGIAAIACIAC	A(A/G)IGCTA(A/G)IGGIA(A/G)ICC	P-loop;GLPL
FR4	GGTGGGGTTGGGAAGACAACG	CACGCTAGTGGCAATCC	P-loop;GLPL
FR5	CCGGGITCAGGIAARACWAC	CCCGAAGGAAACCRISRACWARA	P-loop /hydrophobic domain
FR6	GGIGGIGTIGGIAA(A/G)ACIAC	A(A/G)IGCIA(A/G)IGGIA(A/G)ICC	NBS-LRR/P-loop
FR16	GGWATGGGWGGWRTHGGWAARACHAC	ARNWYYTTVARDGCVARWGGVARWCC	
FR19	GGNGGNRTNGGNAARACCAC	CAANGCCAANGGCAANCC	P-loop /hydrophobic domain
FR20	GGTGGGGTTGGGAAGACAACG	CAACGCTAGTGGCAATCC	NBS-LRR/P-loop:NBS-LRR
FR21	GGNGTNGGNAARACNAC	ARIGCTARIGGIARICC	P-loop;GLPL9S/A)L
FR23	GGIGGIGTIGGIAAIACIAC	ARIGCTARIGGIARICC	NBS-LRR/P-loop;NBS-LRR

3.4 Sequencing and Phylogenetic analysis.

The PCR amplification products were cloned and sequenced at Bioserve Biotechnologies (INDIA) Pvt. Ltd., Hyderabad. The sequence data were subjected to GenBank searches with BLAST (Altschul et al. 1990) [50] and BLASTN algorithm via the National Centre for Biotechnology Information (NCBI) website. Multiple alignment of the nucleotide and amino acid sequences were performed using Clustal Omega program of EMBL-EBI. Phylogenetic analyses were performed using MEGA7 software and a Neighbor joining tree based on DNA sequence CLUSTALW alignment of the resistance gene candidates were constructed. Robustness of clustering was checked by bootstrapping 1000 replicates. ORF Finder was used to find the ORF in the DNA sequence (www.ncbi.nlm.nih.gov/orffinder/).

3.5 RNA isolation and RT-PCR analysis.

Zingiber RGC-specific primer pairs were deduced from the RGCs isolated from the amplified *Zingiber spp*. using the software Primer-Blast in NCBI. Altogether 10 RGC-specific primers were designed (Table 2). Using these primers, conditions for PCR amplification were standardized using genomic DNA. Total RNA was isolated from young leaves collected from infected fields using RNA isolation kit (RNeasy Mini Kit, QIAGEN). Total RNA was treated with DNase I to remove any traces of genomic DNA. The RNA was treated with DNase I (Promega, USA) for 1 h to remove DNA contamination. The RT-PCR reactionswere performed using One Step RT PCR kit (Invitrogen) following the instructions. The reaction included a positive control with Actin specific primers and a negative control without RNA. The reaction conditions were 5 min at 94^{O}C, followed by 35 cycles of denaturing at 94^{O}C for 1 min, annealing at 55-57^{O}C for 30 sec, and elongating at 72^{O}C for 2 min followed by a final extension at 72^{O}C for 7 min. Amplicons were separated on a 1.2% agarose gel.

Table 2: List of RGC specific primers designed using NCBI Primer Blast software

Sl no.	Primer	Sequence (5' → 3')	
		Forward	Reverse
1	RSP1	AGTCATGGTGTTCACGACCC	CTGAGGGGAGAAGATCCCCA
2	RSP2	ACCACTGCAGGACAGTGATG	GCTTCTGGCCTTGCTCAGTA
3	RSP3	AGGCTGACATGAAAGGGCTC	GAGGCGTGCGCATTCTTTAG
4	RSP4	GCAGGCAAAAGAAAGGCTCC	GGCCTGCCATTTTTCAGCAA
5	RSP5	GAGAGGAGTGGTGTTGGGTG	TCCCCATCGTTGTTCTGCTC
6	RSP6	CCAGCATTCGAGGGGAGAAC	GGATGGCACACTCGGCTATT
7	RSP7	GCAGTGTGTGCAGTCCTAGA	CGTGTCATTTGGGTTGTGGC
8	RSP8	CAGCCCCTTAAAGTCGTGGT	ACATCCCCATCGTGGTTCTG
9	RSP9	GGATGGCACACTCGGCTATT	ATCACACGATGTCTCGCTGG
1	RSP10	TGGAGCAGTCTTTTGGTGGC	CCTGCAGTGTGTTCAGTCCT

4. Result and Discussion

4.1 Amplification of RGCs from *Zingiber* spp.

Using the 10 resistance gene specific degenerate primers, PCR products were obtained from genomic DNA templates of *Zingiber* spp.The amplification products were visualized following electrophoresis in 1.8% agarose gel (Sigma Aldrich Chemicals Pvt. Ltd., India) in 0.5X TBE (10X stock contained 1 M Tris, 0.8 M boric acid, 0.5 M EDTA), and staining with ethidium bromide (0.5 mg=ml). The gels were photographed under a gel documentation system (Perkin Elmer Geliance 200). PCR amplification resulted in the productionof major band in the expected size range of ~ 600bp and ~ 350bp as reported in the literature for other plant species after amplification at 55 °C annealing temperature (Fig. 1 & Fig. 2).

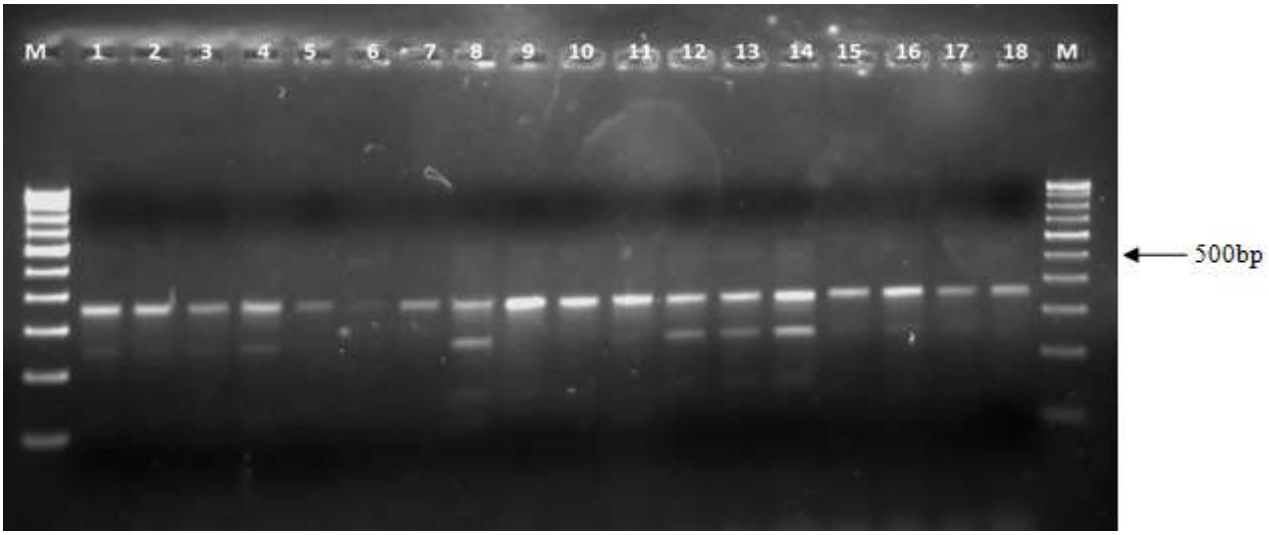

Figure 1: PCR amplification products of 350bp generated by the RGC specifc degenerate primer pairs FR19 in different cultivars of *Zingiber* spp. lane M- 100bp ladder; lane1- *Z. officinale* var. Meitei shing; lane 2- *Z. zerumbet*; lane 3- *Z. Roseum*; lane 4- *Z. officinale* bht; lane 7- *Z. officinale* bpt; lane 9- *Z. sp* 74; lane 10- *Z. sp* 101, lane11- *Z. zerumbet* 126; lane 12- *Z cassumnar* 12; lane 13- *Z. zerumbet* 42; lane 14- *Z. zerumbet var.* Darceyi Lane15- T3 *Z. montanum*; lane 16- T6 *Z. montanum*; lane 17- *Z. officinale* ms; lane 18- T5 *Z. officinale*

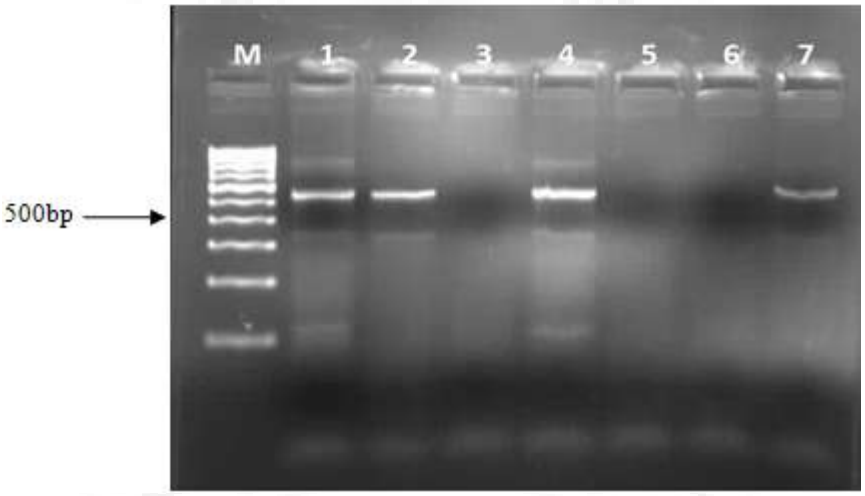

Figure 2: PCR amplification products of 600bp generated by the RGC specifc degenerate primer pairs FR6 in different *Zingiber* spp. lane M- 100bp ladder; ; lane 1- *Z. zerumbet*; lane 2- *Z. zp* 101; lane4- *Z. zerumbet var.* Darceyi; lane7- *Z. cassumnar* 12

4.2 Sequence characterisationand phylogenetic relationships of *Zingiber* RGCs

The 16 selected sequence data were subjected to GenBank searcheswith BLAST [50] and BLASTN algorithm via the National Centre for Biotechnology Information (NCBI) website. No significant similarity was found in 4 RGC sequence data with the databases in the GenBank. The amplification of such unrelated sequences may be due to the amplification on basis of P-loop alone [51]. Remaining RGC sequences showed a high level of sequence identity to comparable regions of disease resistance genes in GenBank, supported by low e-values (Table 3). The level of sequence identity between *Zingiber* RGC sequences and known resistance genes in the top blast hits varied from 87% to 91% between Zne19p6 and Zne31p6 respectively to Zingiber officinale clone ZoP26(e-value: 1e-106) and Zingiber zerumbet clone ZzP226(e-value: 1e-81).BLASTP analysis in the genebank database of deduced amino acid sequences revealeddetection of putative conserved domains of superfamilies NS-ARC,significant homology to well characterised R-genes from other plant species and similarity to putative disease resistance proteins. The presence of NB-ARC domainshows the amino acid sequences to be analogous to plant R-gene products. The RGCs possess conserved amino acid motifs along the NBS sequence which includes the P-loop and the kinase-2 domains, which are ATP- and GTP-binding sites [44] [45].Out of the 16 deduced amino acid sequences 7 sequences were unrelated to resistance genes. Further analysis of the sequences using ORF Finder at NCBI server revealed that all the16 sequences could be translated into a single open reading frame (ORF) of length ranging from 100 amino acids to 138 amino acids. Further analysis of these 16 RGCs revealed the presence of stop codons in 13 out of the 16 *Zingiber* RGCs.

Table 3: Similarity of *Zingiber* RGC to accessions within GenBank using BLASTN

S no.	RGC	Blast top hits, organism, Description	Identities	e-value
1	zne10p6	*Zingiber officinale* clone ZoP26 CC-NBS-LRR disease resistance protein-like gene, partial sequence	89%	3e-124
2	zne19p6	*Zingiber officinale* clone ZoP26 CC-NBS-LRR disease resistance protein-like gene, partial sequence	87%	1e-106
3	zne30p6	*Zingiber zerumbet* clone ZzP29 CC-NBS-LRR disease resistance protein-like gene, partial sequence	90%	1e-77
4	zne31p6	*Zingiber zerumbet* clone ZzP226 CC-NBS-LRR disease resistance protein-like gene, partial sequence	91%	1e-81
5	zne23p6	*Zingiber zerumbet* clone ZzP226 CC-NBS-LRR disease resistance protein-like gene, partial sequence	89%	2e-150
6	zne24p6	*Zingiber zerumbet* putative CC-NBS-LRR disease resistance protein gene, partial sequence	91%	1e-156

Multiple alignment of the nucleotide sequences and deduced amino acid sequences were performed using Clustal Omega program of EMBL-EBI (Fig. 3).The amino acid alignment showed homology of Zingiber RGCs with targeted NBS-LRRdomains of well characterized R genes from other plants. NBS-LRR domain is found to be the largest class of plant R-genes. Around 150 genes in the genome of *Arabidosis thaliana* are reported to to code for NBS-LRR motifs [10]. Such a wide prevalence of the NBS-LRR gene signifies their ancient origin [8]. Moreover, several features of the RGC sequences isolatedshows that RGC sequences belong to non-TIR NBS-LRR class of resistance gene.In this study no TIR type sequences were found as have been reported from earlier similar works [45] [35] [52] [53]. It seems in the earlier studies, TIR domain has not been reported in the NBS-LRR R-genes of other monocots such as in wheat [54], rice [55] [11] and maize [56].

Multiple sequence alignment Result

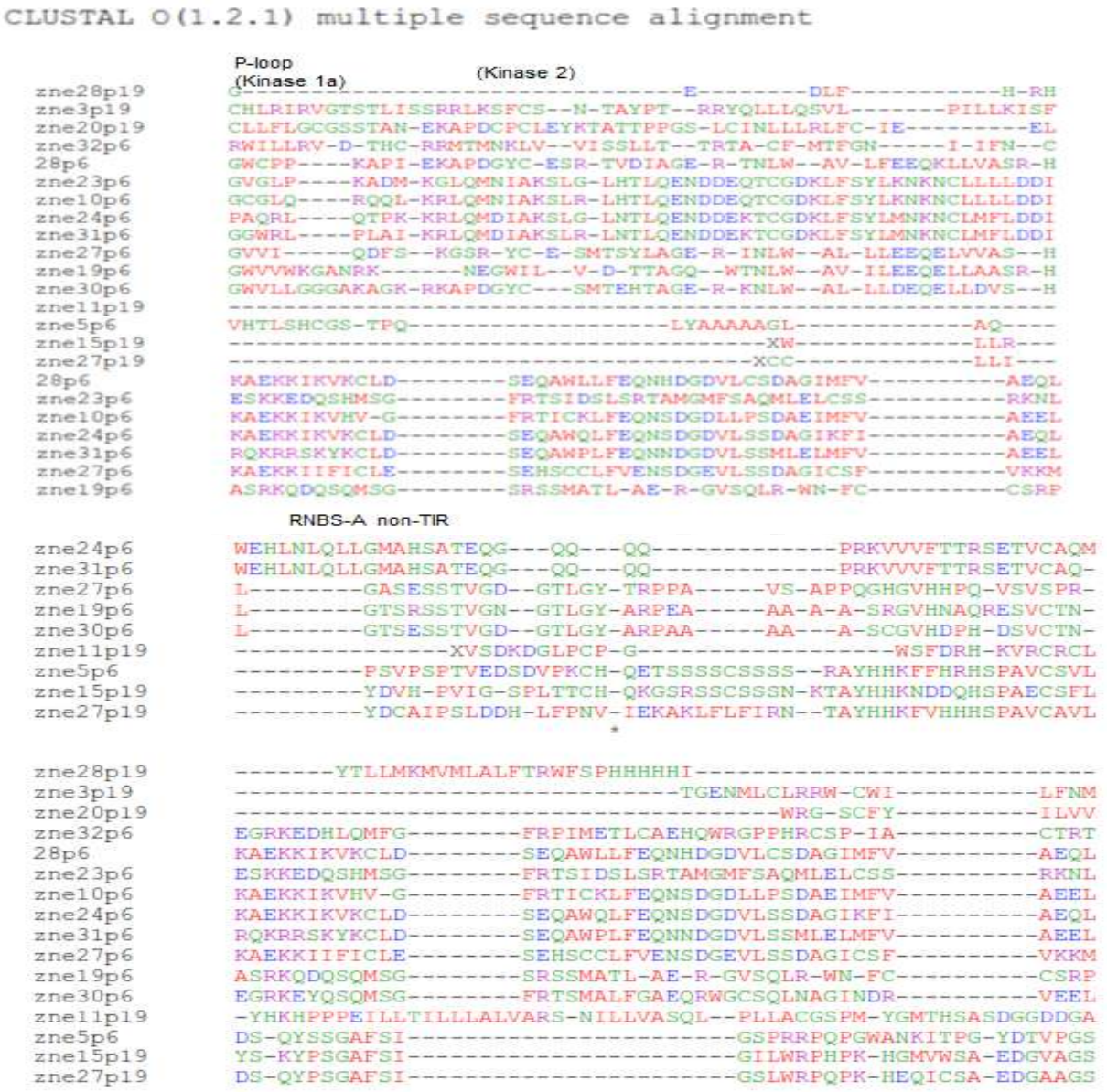

Figure 3: Multiple amino acid sequence alignment of representative *Zingiber* RGCs with NBS domains of R-genes using the CLUSTAL Omega program of EMBL-EBI

Phylogenetic analyses were performed usingMEGA7 softwareand aNeighbor joining tree based on amino acid CLUSTALW alignment of the resistance gene candidates were constructed (Fig. 4). It was carried out to examine the relationships of *Zingiber* resistance gene candidates (RGCs) among themselves and to R-genes from other plant species. Robustness of clustering was checked by bootstrapping1,000 replicates and bootstrap values are given at the branch points. The data revealed moderate to high diversity in the collection, clustering them into four major phylogenetic groups (A-D).The *Zingiber* RGCs consists of non TIR NBS-LRR disease resistance proteins. Group A consist of 9 RGCs, group B consist of 7 RGCs, group C and D consist of 3 RGC sequences each.In group A,sequences of CC NBS LRR class were clustered together.Further, all the groups i.e., A-D is clustered into two sub-clusters each. Group B comprises RGC sequences where no putative conserved domains have been detected. The sequences identified in group A can be treated as resistance gene candidates (RGCs) based on their high level of sequence identities to known R-genes from other species, considerably long open reading frames and presence of conserved motifs characteristic of NBS-LRR R genes. The phylogenetic result shows that *Zingiber* RGCs mainly comprised of CC-NBS-LRR class of disease resistance gene.

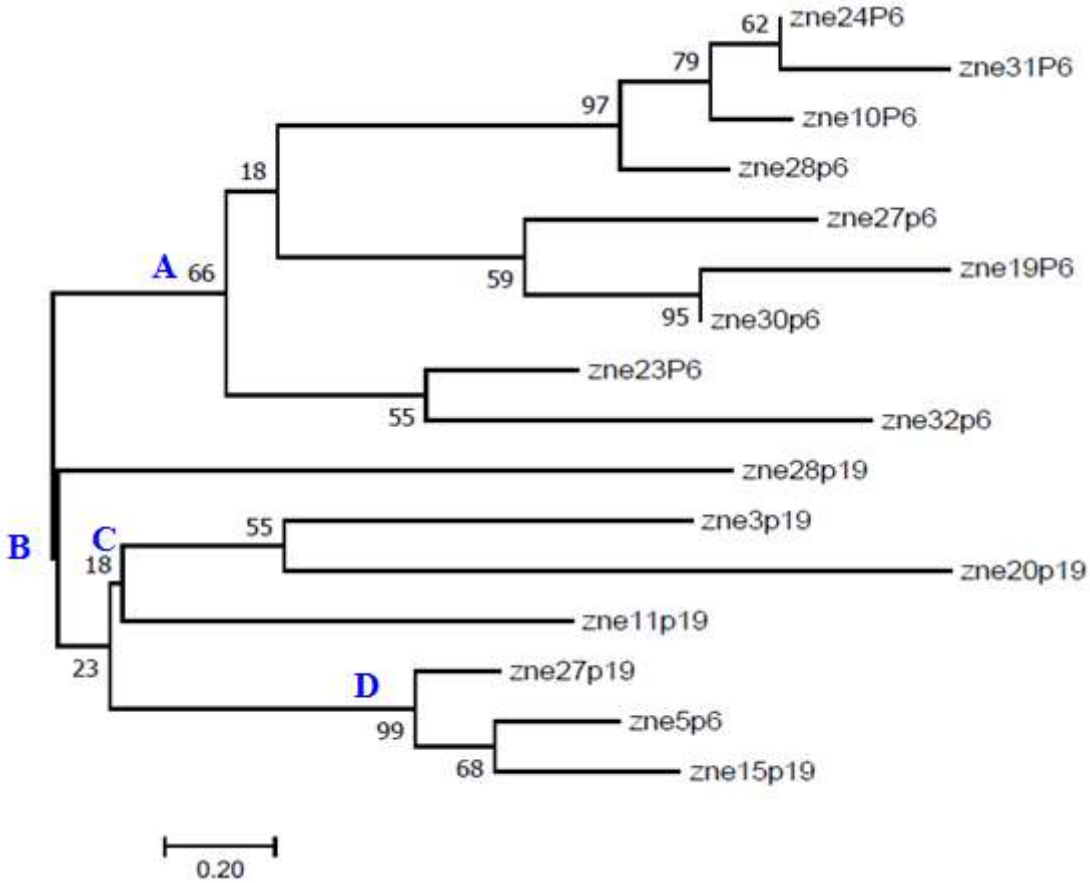

Figure 4: Neighbor joining tree based on CLUSTALW alignment of amino acid sequences of resistance gene candidate of *Zingiber* spp. collected from North-East India and NBS sequences of R-genes from other plant species.. Bootstrap values are given at the nodes and the corresponding RGCs clustering together are indicated. Bootstrap values 1000 and the scale of genetic distance as computed from the pairwise distance in CLUSTALW are indicated. Four phylogenetic groups have been identified (A-D)

4.3 Expression analysis

Using the 10RGC specific primers designed to *Zingiber* RGCs, PCR was carried out with genomic DNA of the 14 accessionsof *Zingiber* spp. in which predicted PCR amplification product was detected with resistance genedegenerate primers. The 10 RGC specificprimers yielded PCR products of the predicted size from the genomic DNA. However, when RT-PCR was conducted with the primers designed,only two primers i.e., RSP1 and RSP3 were found to yield amplification products of 300bp and 200 bp respectively (Fig.5& Fig.6). These results show that the RGCs failed to produce a transcript for disease resistance. The lack of expression might be due to presence of some non-functional promoter preceding the sequences or due to expression of low transcript levels theywere not expressed orthe RGCs might correspond to pseudogenes. It has been reported earlier that the majority of the NBS-LRR resistance genes are generally expressed at a low level [57].Expression of R-genes has been found in highly resistant varieties, but not in partially resistant varieties [58].Both the amplification products of 300 bp and 200 bp were gel purified, cloned and sequenced at Bioserve technologies. The sequence data were used for homology searches and BLASTP analysis.The RT-PCR products amplified were not related with disease related proteins and did not show any significant similarity with the R-genes of other plant species. They were uncharacterised proteins.

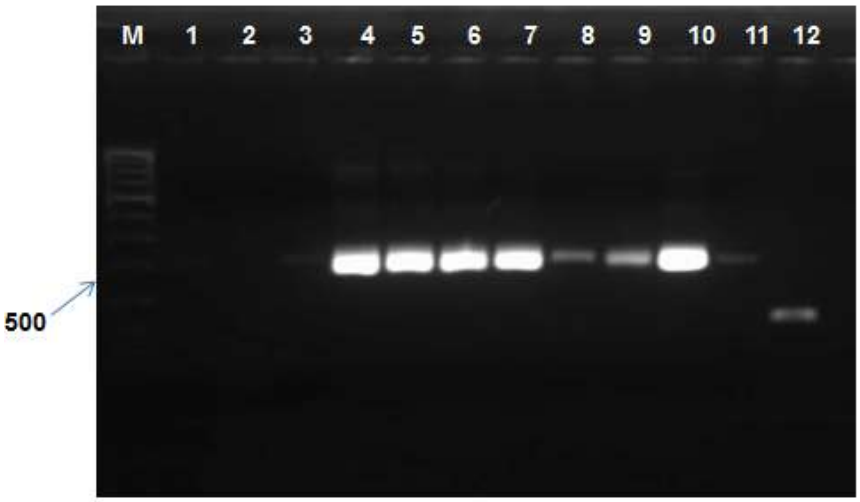

Figure 5: PCR amplification products of 300bp generated by the RGC specific primer designed RSP1; lane M- 100bp ladder; lane 4- *Z. officinale* makheer; lane 5- *Z. zerumbet*; lane 6- *Z. zerumbet* 126; lane 7- *Z. sp* 101; lane 8- *Z. officinale* maran; lane 9- *Z. officinale* MSa; lane 10- *Z. zerumbet var.* Darceyi; lane 12- +ve Control

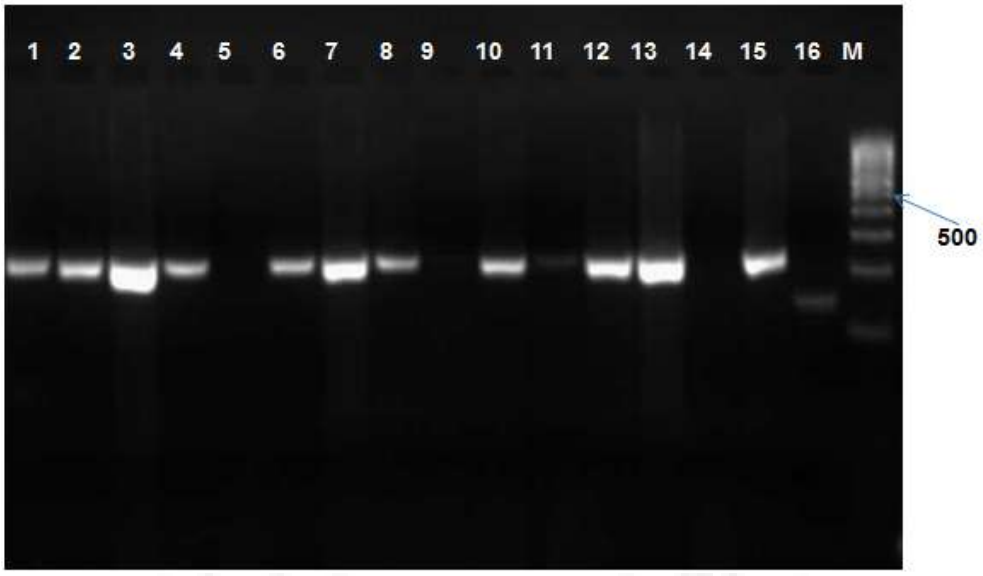

Figure 6: PCR Amplification products of 200bp generated by the RGC specific primer designed RSP3; lane1- *Z. officinale* var. Meitei shing; lane 2- *Z. zerumbet*; lane 3- *Z. cassumnar* 12; lane 4- T6 *Z. montanum*; lane 6- . *zerumbet* var. Darceyi; lane 7- *Z. sp* 101; lane 8- *Z. officinale* bar ; lane10- *Z. officinale* ZRL; lane 12- *Z. zerumbet* 126; lane 13- *Z. zerumbet* var. Darceyi 40; lane 15- *Z. officinale* Hei; lane 16- +ve control; lane M- 100bp ladder

5. Conclusion & Future Scope

We can summarize thatrhizome rotor *Fusarium*yellows caused by the fungus *Fusarium oxysporum* f. sp. *Zingiberi* is found to be prevalent in the Northeastern region of India and till now no work has been done or reported to isolate and characterise the *Fusarium* resistance gene candidatesfrom the *Zingiber* spp.of the region.This study reports for the first time on characterization and molecular analysis of *Fusarium* resistance gene candidates from *Zingiber* spp. of North east India. From the results, we can conclude that RGCs were detected in PCRusing resistance gene degenerate primers as well as the RGC-specific primers designed. Thus, we can state that degenerate primers can be used to isolate RGCs from plant species. *Zingiber* RGCs belong to the CC-NBS-LRR class of proteins. While no NBS-LRR-TIR class of R-gene is detected in the study. BLASTP analysis of the amino acid sequences of the RGCs shows presence of conserved motifs of superfamilies NS-ARC and significant homology with R-genes in the GenBank database from other plant species. However, the expression analysis reveals thatthe RGCs were expressed ata very low level to be detected or they fail to express in the *Zingiber* species found in the region. TheRGCs can be further analyzed and used to study the organization and functioning of the R-genes belonging to the NBS-LRR class in asexually reproducing plants. The RGCs can also be used in characterizing R-genes from related plant species.

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