

Original Research Article

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Isolation and Characterisation of *Fusarium* Resistance Gene Candidates in *Zingiber* spp. of North East India

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ABSTRACT

Rhizome rot and yellows caused by *Fusarium oxysporum* f. sp. *Zingiberi*, is a serious a serious soil-borne disease of ginger and found to be prevalent in the north-east region of India. Most of the plant disease resistance (R) genes encode a highly conserved nucleotide binding site and leucine-rich repeat structure (NBS-LRR). These structure can be used to isolate candidate genes for *Fusarium* resistance in *Zingiber* spp. Degenerate oligonucleotides which have been designed to recognize conserved coding regions of known resistance (R) genes from different species of plant in literature were obtained to target PCR to amplify resistance gene analogs (RGAs) from *Zingiber* spp. of north east India. PCR amplification from genomic DNA yielded a group of fragments of approximately 350bp and 600bp DNA sequences. From the results we can conclude that *Zingiber* RGCs belong to the CC-NBS-LRR class of proteins and no TIR-RGCs were found in the study. Although RGCs were detected with degenerate RGC primers and the RGC-specific primers we designed. The expression of the disease resistance seems to be very low or absent in the *Zingiber* species found in the region.

Keywords

R-genes, Resistance gene candidates (RGC), CC-NBS-LRR class, *Fusarium oxysporum* f. sp. *Zingiberi*

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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. It is estimated that more than 50% of the national production ginger comes from the North Eastern States. Ginger is used throughout the world as a spice or fresh herb or in medicines.

Cultivated ginger originated in India or Southeast Asia (Ravindran *et al.*, 1994). Globally, the main producers of ginger are India, China, Nepal, Indonesia and Nigeria (FAOSTAT 2010). 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 (FAOSTAT data, 2014). Ginger is grown in almost all the states of the North-Eastern region of India. Assam ranks first in ginger acreage as well as in production but productivity was highest in Mizoram, followed by Arunachal Pradesh, Assam and

Nagaland (H Rahman *et al.*, 2009). 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 like Nadia and Rio-De-Janeiro but have more fibre 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, climate and other ecological factors of the region enormously favours the growth and development of the crop and there is a tremendous scope to develop for increase in its yield per unit area. As such the farmers here are interested for the cultivation of the crop. But it seems the continuous domestication of preferred genotypes of ginger and their exclusive vegetative propagation have resulted in the degradation of the genetic base of this crop and 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 (Stirling, 2004). Rhizome rot is found to be prevalent in many areas of north-east India as well. As ginger is an obligatory asexual crop, resistance breeding is limited only to its germplasm screening (Ravindran *et al.*, 2005). Till now no work has been taken upto evaluate the wild relatives of ginger for *Fusarium* wilt resistance in North east India. Therefore, the genetic resources 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) hold much promise to investigate features of resistance-related loci in ginger for its genetic improvement.

Plant disease resistance genes (R-genes) are found to be an important component of the genetic resistance mechanism in plants (Flor 1971; Dangl and Jones 2001). R-genes have a key role in recognizing proteins expressed by specific a virulence (Avr) genes of pathogens (Flor 1956). The NBS-LRR R-genes seems to be abundant in plant genomes with approximately 150 and 600 isolated from *Arabidopsis* and rice respectively (Meyers *et al.*, 2003; Zhou *et al.*, 2004).

About 75% of plant R-genes encode proteins with a nucleotide-binding site and leucine-rich repeat (NBS-LRR) domains that conferring resistance to various pest and pathogens such as bacteria, fungi, viruses, insects and nematodes (Dangl and Jones, 2001). 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 (Belkhadir *et al.*, 2004).

Wild relatives of many other plants have been used as an important source of genetic variation for disease resistance (Xiao *et al.*, 1998; Zamir, 2001) since they can evolve resistance specificities more efficiently than cultigen (Clay and Kover, 1996; Ebert and Hamilton, 1996). 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.

Materials and Methods

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 where the cultivars are maintained as accessions under shade house conditions.

Apart from these, *Zingiber* spp. spreading across different locations of north east from farmer's fields were collected 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.

Isolation of genomic DNA

Total genomic DNA was extracted from young leaves using CTAB method using the procedure of Doyle and Doyle (1987) with minor modifications. DNA was diluted to 20 ng/μl final concentration in sterile deionised water and stored in 1X TE buffer (10 mM Tris HCl pH 8, 1 mM EDTA pH 8) at -20 °C. The quantity and quality of DNA preparation were verified by standard spectrophotometry methods (Nanodrop Spectrophotometer ND 2000) and visualized on 0.8% agarose gel stained with ethidium bromide.

Primers and PCR amplification

A Total of 10Resistance gene specific degenerate primers (Table 1, Sigma Aldrich Chemicals Pvt. Ltd., India) previously used in published literature for amplifying RGCs in other crops were 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 Master cycler pro S programmed for an initial denaturation at 94°C for 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 final extension step at 72°C for 5 min.

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) and BLASTN algorithm via the National Centre for Biotechnology Information (NCBI) web site. Multiple alignment of the nucleotide and amino acid sequences were performed using Clustal Omega program of EMBL-EBI. Phylogenetic analyses were performed using MEGA6 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/) (Table 2).

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. 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 (RN easy 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 reactions were 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°C, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 55-57°C for 30 sec, and elongating at 72°C for 2 min followed by a final extension at 72°C for 7 min. Amplicons were separated on a 1.2% agarose gel..

Results and Discussion

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 production of 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 and 2).

Sequence characterisation and phylogenetic relationships of *Zingiber* RGCs

The 16 selected sequence data were subjected to GenBank searches with BLAST (Altschul *et al.*, 1990) 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 (Rigden *et al.*, 2000). 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 revealed detection of putative conserved domains of super families 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 domain shows the amino acid sequences to be analogous to plant R-gene products. 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 the 16 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.

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-LRR domains 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 *Arabidopsis thaliana* are reported to code for NBS-LRR motifs (Meyers *et al.*, 2003). Such a wide prevalence of the NBS-LRR gene signifies their ancient origin (Dangl and Jones, 2001). Moreover, several features of the RGC sequences isolated shows that RGC sequences belong to non-TIR NBS-LRR class of resistance gene. In this study also no TIR type sequences were found as have been reported from earlier similar works (Meyers *et al.*, 1999; Pan *et al.*, 2000; Cannon *et al.*,

2002: Joshi *et al.*, 2012). It seems in the earlier studies, TIR domain have not been reported in the NBS-LRR R-genes of other monocots such as in wheat (Dilbirligi and Gill, 2003), rice (Monosi *et al.*, 2004; Zhou *et al.*, 2004) and maize (Xiao *et al.*, 2006).

Phylogenetic analyses were performed using MEGA7 software and a Neighbor 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 bootstrapping 1,000 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 of NBS LRR class were clustered together. Further, all the groups i.e., A-D is clustered into two sub-cluster 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.

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 GGTGGGAA 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	CCCGAAGGAAACCRISRACWAR A	P-loop /hydrophobic domain
FR6	GGIGGIGTIGGIAA(A/G)ACIAC	A(A/G)IGCIA(A/G)IGGIA(A/G)ICC	NBS-LRR/P-loop
FR16	GGWATGGGWGGWRTHGGWA ARACHAC	ARNWYYTTVARDGCVARWGGV ARWCC	
FR19	GGNGGNRTNGGNAARACCAC	CAANGCCAANGGCAANCC	P-loop /hydrophobic domain
FR20	GGTGGGGTTGGGAAGACAACG	CAACGCTAGTGGCAATCC	NBS-LRR/P-loop; NBS-LRR
FR21	GGNGTNGGNAARACNAC	ARIGCTARIGGIARICC	P-loop; GLPL9S/A)L
FR 23	GGIGGIGTIGGIAAIACIAC	ARIGCTARIGGIARICC	NBS-LRR/P-loop; NBS-LRR

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	GCAGGCAAAGAAAGGCTCC	GGCCTGCCATTTTTTCAGCAA
5	RSP5	GAGAGGAGTGGTGTGGGTG	TCCCCATCGTTGTTCTGCTC
6	RSP6	CCAGCATTTCGAGGGGAGAAC	GGATGGCACACTCGGCTATT
7	RSP7	GCAGTGTGTGCAGTCCTAGA	CGTGTCATTTGGGTTGTGGC
8	RSP8	CAGCCCCTTAAAGTCGTGGT	ACATCCCCATCGTGGTTCTG
9	RSP9	GGATGGCACACTCGGCTATT	ATCACACGATGTCTCGCTGG
1	RSP10	TGGAGCAGTCTTTTGGTGCC	CCTGCAGTGTGTTTCAGTCCT

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

Sl no.	RGC	Blast top hits, organism, Description	Identities	e-value
1	zne10p6	<i>Zingiber officinale</i> cloneZoP26 CC-NBS-LRR disease resistance protein-like gene, partial sequence	89%	3e-124
2	zne19p6	<i>Zingiber officinale</i> cloneZoP26 CC-NBS-LRR disease resistance protein-like gene, partial sequence	87%	1e-106
3	zne30p6	<i>Zingiber zerumbet</i> cloneZzP29 CC-NBS-LRR disease resistance protein-like gene, partial sequence	90%	1e-77
4	zne31p6	<i>Zingiber zerumbet</i> cloneZzP226 CC-NBS-LRR disease resistance protein-like gene, partial sequence	91%	1e-81
5	zne23p6	<i>Zingiber zerumbet</i> cloneZzP226 CC-NBS-LRR disease resistance protein-like gene, partial sequence	89%	2e-150
6	zne24p6	<i>Zingiber zerumbet</i> putative CC-NBS-LRR disease resistance protein gene, partial sequence	91%	1e-156

Fig.1 PCR amplification products of 350bp generated by the RGC specific degenerate primer pairs FR19 in different cultivars of *Zingiber spp.* lane M- 100bp ladder; lane 1-*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, lane 11-*Z. zerumbet*126; lane 12- *Z cassumnar* 12; lane 13- *Z. zerumbet* 42; lane 14- *Z. zerumbet* var. Darceyi Lane 15- T3 *Z. montanum*; lane 16- T6 *Z. montanum*; lane 17- *Z. officinale* ms; lane 18- T5 *Z. officinale*

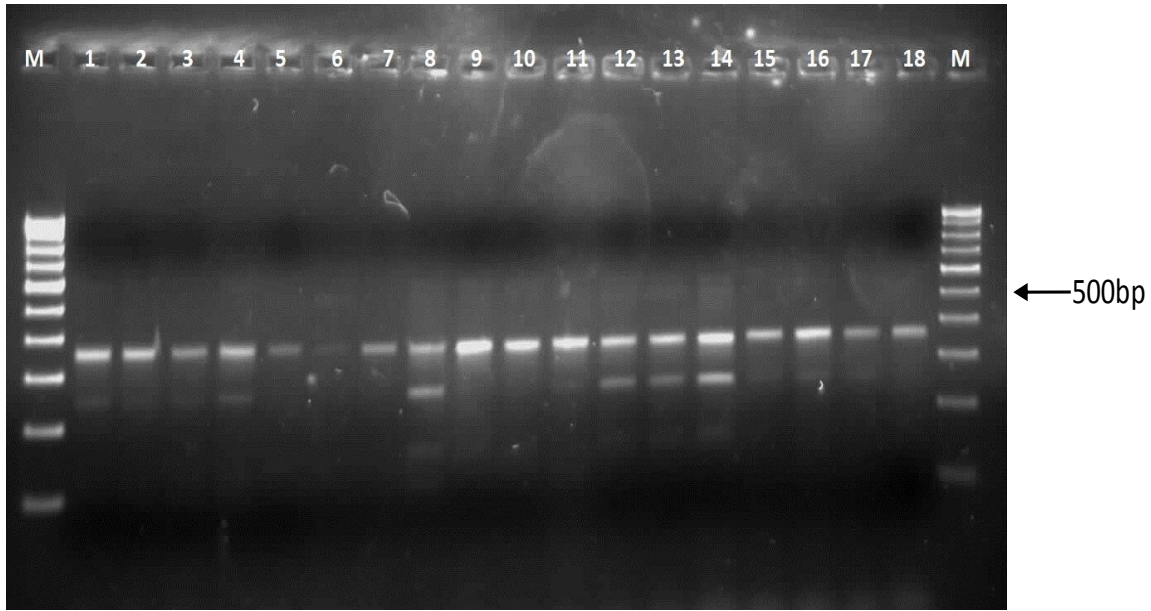


Fig.2 PCR amplification products of 600bp generated by the RGC specific degenerate primer pairs FR6 in different *Zingiber spp.* lane M- 100bp ladder; ; lane 1- *Z. zerumbet*; lane 2- *Z. zp* 101; lane 4- *Z. zerumbet* var. Darceyi; lane 7- *Z. cassumnar* 12

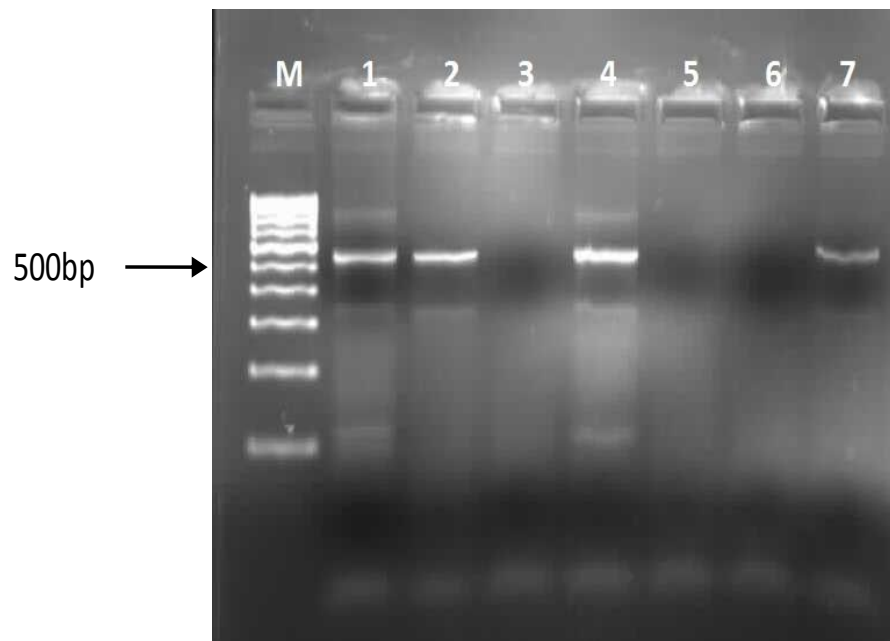
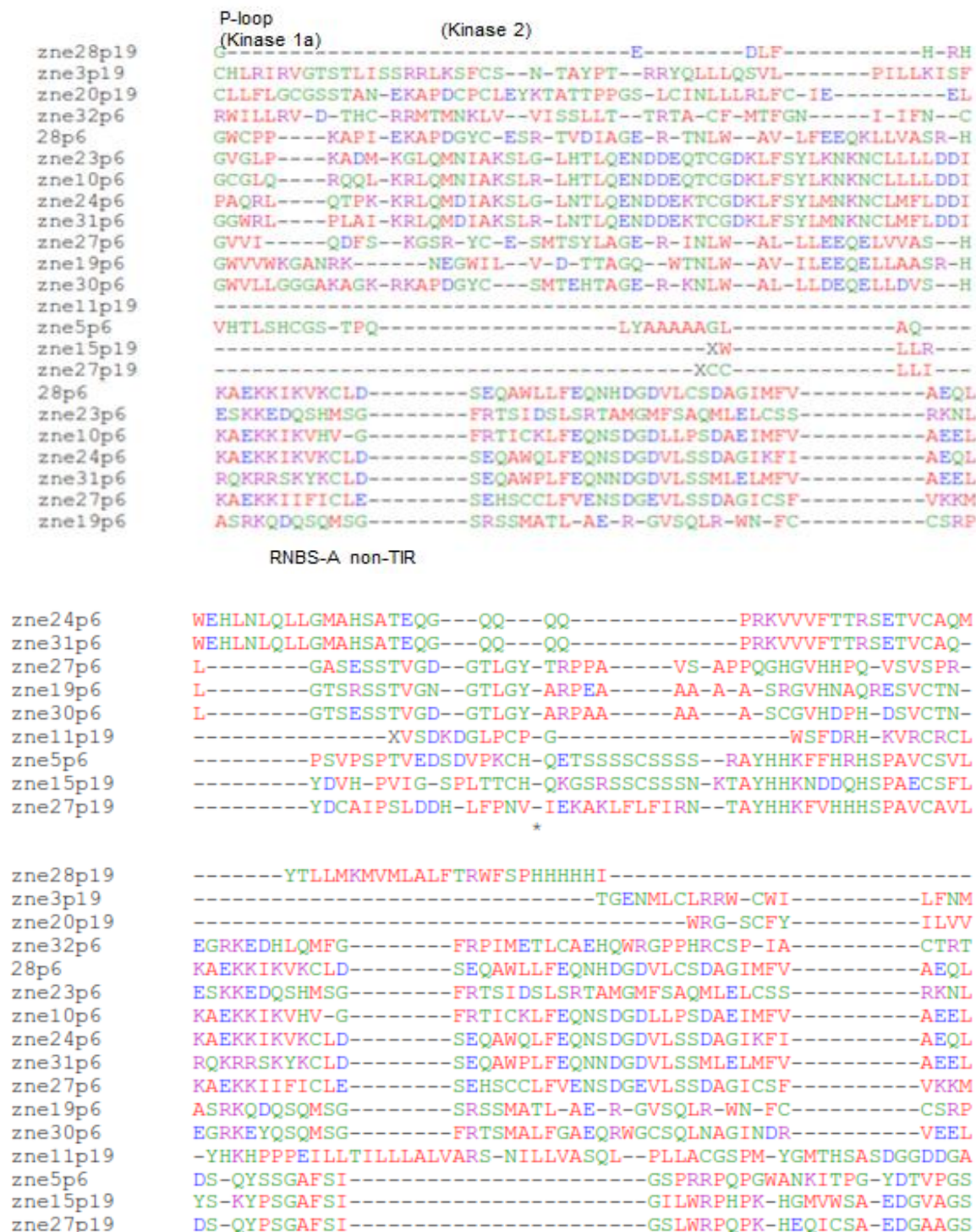


Fig.3 Multiple amino acid sequence alignment of representative Zingiber RGCs with NBS domains of R-genes using the CLUSTAL Omega program of EMBL-EBI

CLUSTAL O(1.2.1) multiple sequence alignment



Multiple sequence alignment Result

Fig.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)

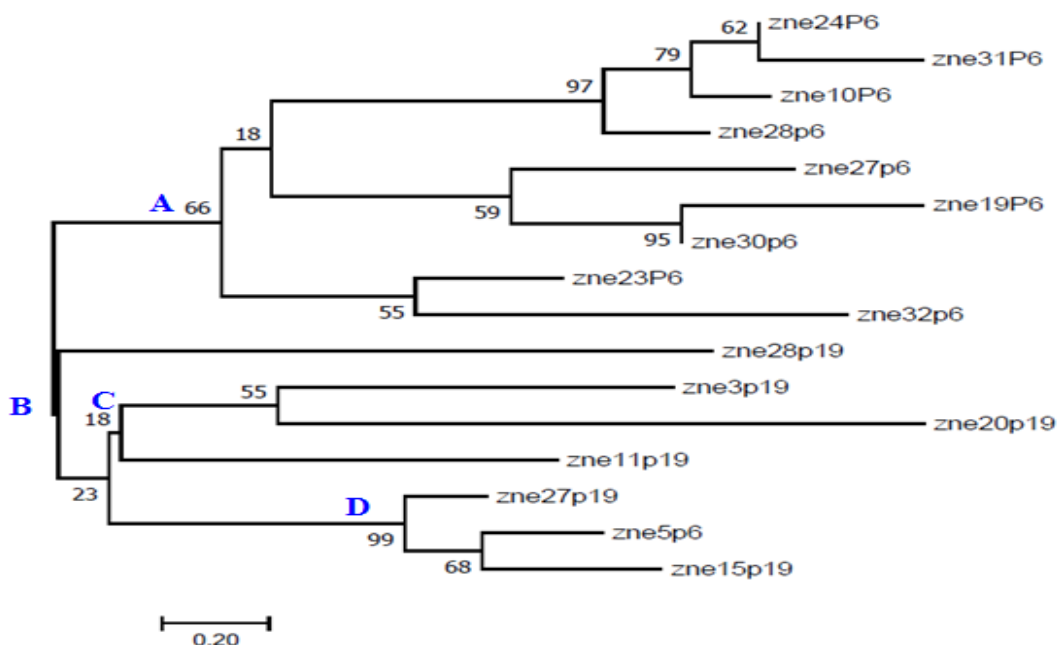


Fig.5 PCR amplification products of 300bp generated by the RGC specific primer pair designed 10p6; lane M- 100bp ladder; lane 4- *Z. officinale* makheer; lane 5- *Z. zerumbet*; lane 6- *Z. zerumbet* 126; lane 7- *Z. sp* 101; lane 8- *Z. officinale* maran; lane 9- *Z. officinale* MSa; lane 10- *Z. zerumbet* var. Darceyi; lane 12- +ve Control

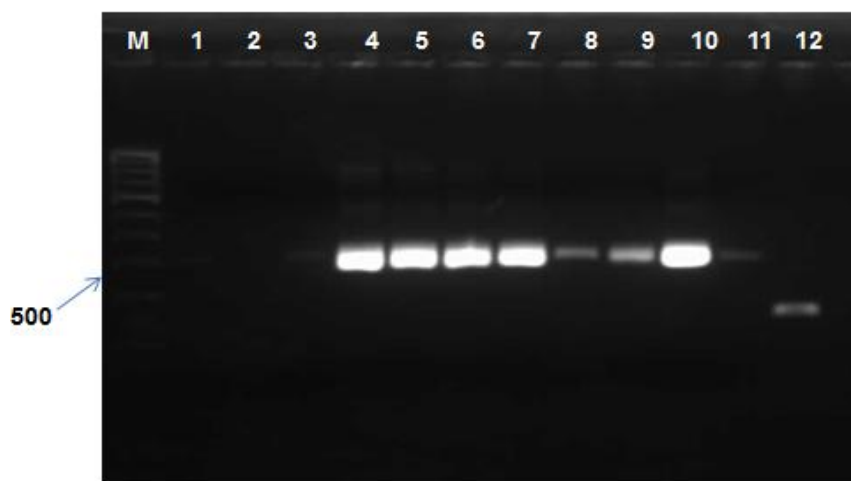
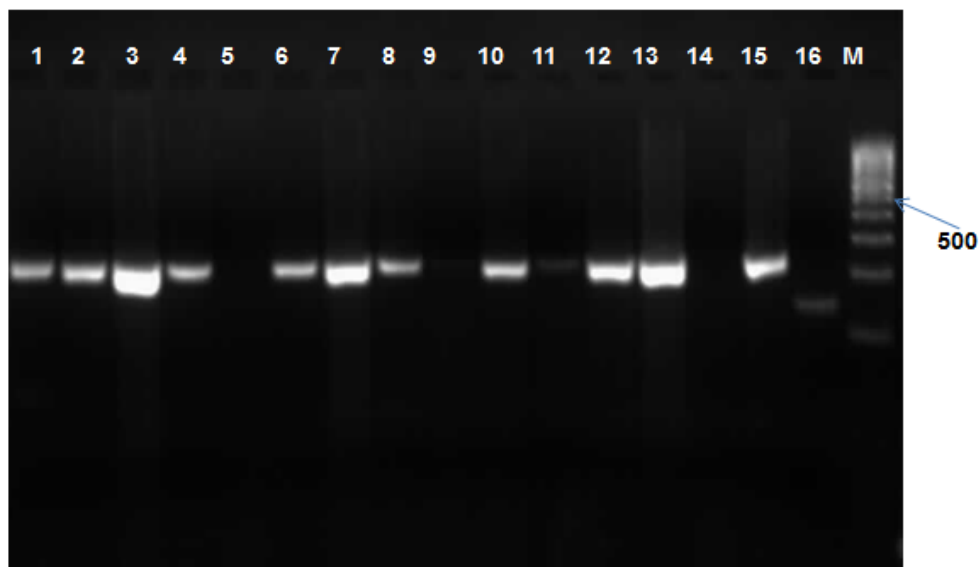


Fig.6 PCR Amplification products of 200bp generated by the RGC specific primer pair designed 23p6; lane1- *Z. officinale* var. Meitei shing; lane 2- *Z. zerumbet*; lane 3- *Z. cassumnar* 12; lane 4- T6 *Z. montanum*; lane 6- *Z. zerumbet* var. Darceyi; lane 7- *Z. sp* 101; lane 8- *Z. officinale* bar ; 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



Expression analysis

Using the 10RGC specific primers designed to *Zingiber* RGCs, PCR was carried out with genomic DNA of the 14 accessions of *Zingiber* spp. in which PCR amplification product is detected with disease resistance degenerate primers. The 10 primers yielded PCR products of the predicted size from the DNA. When RT-PCR was conducted with the 10RGC specific primer pairs, only two primer pairs i.e., RSP1 and RSP3 were found to yield amplification products of 300bp and 200 bp respectively (Fig. 6 and 7). 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 were not expressed or the RGCs might correspond to pseudogenes. It has been reported earlier that majority of the NBS-LRR resistance genes are generally expressed at a low level (Hulbert *et al.*, 2001).

Expression of R-genes has been found in highly resistant varieties but not in partially resistant varieties (Swetha *et al.*, 2008). The amplicons were gel purified, cloned and sequenced at Bioserve technologies. The sequence data were used for homology searches. 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.

In summary, *Fusarium* resistance gene candidates from *Zingiber* spp. have not been isolated and characterised till now from the North-eastern region of India. This study aims at isolating and characterising the RGCs in the region for the first time. From the results we can conclude that *Zingiber* RGCs belong to the CC-NBS-LRR class of proteins and no TIR-RGCs were found in the study. Although RGCs were detected with degenerate RGC primers and the RGC-specific primers we designed. The expression of the disease

resistance seems to be very low or absent in the *Zingiber* species found in the region.

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References

- Aarts MG, Hekkert B, Holub EB, Beynon JL, Stiekema WJ, Pereira A (1998). Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 11:251–258
- Anderson P, Lawrence G, Morrish B, AyliVe M, Finnegan E, Ellis J (1997) Inactivation of the Xax rust resistance gene M associated with the loss of a repeated unit within the leucine-rich repeat coding region. *Plant Cell* 9:641–651
- Anonymous, (2002). Basic Statistics of North Eastern Region, North Eastern Council, Ministry of Home Affairs, Shillong
- Bai J, Pennill L, Ning J, Lee S, Ramalingam J, Webb C, Zhao B, Sun Q, Nelson J, Leach J, Hulbert S (2002). Diversity in nucleotide binding site-leucine-rich repeat genes in cereals. *Genome Res* 12:1871–1884
- Baker B, Zambryski P, Staskawicz B and Dinesh-Kumar S P 1997. Signaling in plant-microbe interactions. *Science* 276: 726–733
- Baldi P, Patocchi A, Zini E, Toller C, Velasco R and Komjanc M (2004). Cloning and linkage mapping of resistance gene homologues in apple. *Theoretical and Applied Genetics*. vol. 109, no. 1, p. 231-239
- Belkhadir, Y., Nimchuk, Z., Hubert, D.A., Mackey, D., and Dangl, J.L. (2004). *Arabidopsis* RIN4 negatively regulates disease resistance mediated by RPS2 and RPM1 downstream or independent of the NDR1 signal modulator and is not required for the virulence functions of bacterial type III effectors AvrRpt2 or AvrRpm1. *Plant Cell* 16:2822–2835
- Dangl JL, Jones DG (2001). Plant pathogens and integrated defence responses to infection. *Nature* 411:826–833
- Deng Z, Gmitter FJ (2003). Cloning and characterization of receptor kinase class disease resistance gene candidates in Citrus. *Theor Appl Genet* 108:53–61
- Deng Z, Huang S, Ling P, Chen C, Yu C, Weber C, Moore G, Gmitter FJ (2000). Cloning and characterization of NBS-LRR class resistance- gene candidate sequences in citrus. *Theor Appl Genet* 101:814–822
- Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998). The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* 10:1915–1925
- Dohroo, N.P. 1989. Seed transmission of pre-emergence rot and yellows in ginger. *Plant Dis Res*. 4:73-74
- FAOSTAT (2010). Food and Agricultural commodities production. <http://faostat.fao.org/site/339/default.aspx>. Accessed 31 Aug 2010
- FAOSTAT Citation database results (2005). Food and Agricultural Organization, Rome. <http://faostat.fao.org> Cited 01 July 2006
- Ferrier-Cana E, GeVroy V, Macadre C, Creusot F, Imbert-Bollere P, Sevignac M, Langin T (2003). Characterization of expressed NBS-LRR resistance gene

- candidates from common bean. *Theor Appl Genet* 106:251–261
- Feuillet C, Schachermayr G, Keller B (1997). Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. *Plant J* 11:45–52
- Flor H.H. (1956). The complementary genetic systems in flax and flax rust. *Adv. Genet.* 8: 29–54
- Flor HH (1971). The current status of gene for gene concept. *Ann Rev Phytopathol* 9:275–296
- Gassmann W, Hinsch ME and Staskawicz BJ (1999). The Arabidopsis RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J.* 20: 265-277
- Grant MR, Godiard L, Straube E, AshWeld T, Lewald J, Sattler A, Innes RW, Dangl JL (1995). Structure of the Arabidopsis RPM1 gene enabling dual specificity disease-resistance. *Science* 269:843–846
- H. Rahman, R. Karuppaiyan, K. Kishore and R. Denzongpa (2009). Traditional Practices of Ginger Cultivation in Northeast India. *Indian Journal of Traditional Knowledge* 8 (1): 23-28
- Hammond-Kosack KE and Jones JD (1997). Plant disease resistance genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48: 575-607
- He CY, Tian AG, Zhang JS, Zhang ZY, Gai JY, Chen SY (2003). Isolation and characterization of a full-length resistance gene homolog from soybean. *Theor Appl Genet* 106:786–793
- Hulbert, S. H., Webb, C. A., Smith, S. M., and Sun, Q. (2001). Resistance gene complexes: Evolution and utilisation. *Annual Review of Phytopathology*, 39, 285-312
- Ilag L L, Yadav R C, Huang N, Ronald P C and Ausubel F M (2000). Isolation and characterization of disease resistance gene homologues from rice cultivar IR64. *Gene*, vol. 255, no. 2, p. 245-255
- Irigoyen ML, Loarce Y, Fominaya A, Ferrer E (2004). Isolation and mapping of resistance gene analogues from the *Avena strigosa* genome. *Theor Appl Genet* 109:713–724
- Johal G, Briggs S (1992). Reductase activity encoded by the HM1 disease resistance gene in maize. *Science* 258:985–987
- Joshi RK, Kar B, Mohanty S, Subudhi E, Nayak S (2012). Molecular cloning, characterization, and expression analysis of resistance gene candidates in *Kaempferia galangal* L. *MolBiotechnol* 50:200-210.
- Kanazin V, Marek LF, Shoemaker RC (1996). Resistance gene analogs are conserved and clustered in soybean. *Proc Natl Acad Sci USA* 93:11746–11750
- Karlin.S. & Altschul. S. F. (1990). *Proc. Nat. Acad. Sci. USA.* 87. 2264-2268
- Kavitha P G and Thomas G (2007). Evaluation of Zingiberaceae for resistance to ginger soft rot caused by *Pythium aphanidermatum* (Edson) Fitzp. *Plant Genet. Resourc. Newslett.* 152 1–4
- Lacock L, Van Niekerk C, Loots S, Du Preez F and Botha A M (2003). Functional and comparative analysis of expressed sequences from *Diuraphis noxia* infested wheat obtained utilizing the conserved Nucleotide Binding Site. *African Journal of Biotechnology*, vol. 2, no. 4, p. 75-81
- Lawrence BM (1984). Major tropical spices—ginger (*Zingiber officinale* Rosc.). *Perfum Flavor* 9:1–40
- Leister D, Ballvora A, Salamini F, Gebhardt C (1996). A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. *Nat Genet* 14:421–428

- Liu J-J, Ekramoddoullah AKM (2003). Isolation, genetic variation and expression of TIR-NBS-LRR resistance gene analogs from western white pine (*Pinus monticola* Dougl Ex D Don.). *Mol Genet Genom* 270:432–441
- Mago R, Nair S, Mohan M (1999). Resistance gene analogues from rice: cloning, sequencing and mapping. *Theor Appl Genet* 99:50–57
- Meyers B, Kozik A, Griego A, Kuang H, Michelmore R (2003). Genome wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell* 15:809–834
- Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW Young ND (1999). Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding super family. *Plant J* 20:317–332
- Nair RA and Thomas G (2007). Evaluation of resistance gene (R-gene) specific primer sets and characterization of resistance gene candidates in ginger (*Zingiber officinale* Rosc.). *Curr. Sci.* 93: 61-66
- Pan Q, Wendel J, Fluhr R (2000). Divergent evolution of plant NBSLRR resistance gene homologues in dicot and cereal genomes. *J Mol Evol* 50:203–213
- Purseglove J W, Brown E G, Green C L, Robbins S R J, (1981). *Spices*, vol. 1. Longman, London, New York, pp. 447–531
- Raabe R D, I L Connors and A P Martinez (1981). Checklist of plant diseases in Hawaii: including records of microorganisms, principally fungi, found in the state. Hawaii Institute of Tropical Agriculture and Human Resources (CTAHR), Information Text Series 022. 313 pp.
- Ravindran P N, Sasikumar B, Johnson K G, Ratnambal M J, Babu K N, Zachariah J T, Nair R R (1994). Genetic resources of ginger (*Zingiber officinale* Rosc.) and its conservation in India. *Plant Genet Resour Newsl* 98:1–4
- Ravindran PN, Babu KN, Shiva KN (2005). Botany and crop improvement of ginger. In: Ravindran PN, Babu KN (eds) Monograph on ginger. CRC Press, Boca Raton pp 15–85
- Rivkin MI, Vallejos CE, McClean PE (1999). Disease-resistance related sequences in common bean. *Genome* 42:41–47
- Selvan MT, Thomas K G and Manojkumar K (2002). Ginger (*Zingiber officinale* Rosc.) in Indian spices: production and utilization (eds) H P Singh, K Sivaraman and M T Selvan (India: Coconut Development Board) pp 110–131
- Shen KA, Meyers BC, Nurul Islam Faridi M, Chin DB, Stelly DM, Michelmore RW (1998). Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. *Mol Plant Microbe Interact* 11:815–823
- Sheo, Govind, Chandra, Ram, Karibasappa G S, Sharma C K and Singh I P, (1998). Research on Spices in NEH Region. ICAR Research Complex for NEH Region, Umiam pp 9-22
- Song WY, Pi LY, Wang GL, Gardner J, Holsten T, Ronald PC (1997). Evolution of the rice Xa21 disease resistance gene family. *Plant Cell* 9:1279–1287
- Stirling A. 2004. The causes of poor establishment of ginger (*Zingiber officinale*) in Queensland, Australia. *Australasian Plant Pathology* 33:203-10
- Swetha Priya, R., R. B. Subramanian (2007). Isolation and molecular analysis of R-gene in resistant *Zingiber officinale* (ginger) varieties against *Fusarium oxysporum* f. sp. *zingiberi*. *Bioresource Technology* 99:4540-4543
- Tang W, Eisenbrand G, (1992). *Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional*

- and Modern Medicine. Springer-Verlag, Berlin, New York, Pp. 1056.
- Traut T W (1994). The functions and consensus motifs of nine types of peptide segments that form different types of nucleotide binding-sites. *European Journal of Biochemistry*. vol. 222, no. 1, p. 9-19
- Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994). The product of the tobacco mosaic virus resistance gene N: similarity to Toll and the interleukin-1 receptor. *Cell* 78:1101–1115
- Xiao WK, Xu ML, Zhao JR, Wang FG, Li JS, Dai JR (2006). Genome wide isolation of resistance gene analogs in maize (*Zea mays* L.). *Theor Appl Genet* 113:63–72
- Zhang J, Guo WZ, Zhang TZ (2002). Molecular linkage map of allotetraploid cotton (*Gossypium hirsutum* L. × *Gossypium barbadense* L) with a haploid population. *Theor Appl Genet* 105:1166–1174
- Zhou T, Wang T, Chen JQ, Araki H, Jing Z, Jiang K, Shen J, Tian D (2004). Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Mol Genet Genom* 271: 402–415

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