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In silico analysis of gall midge resistance gene *Gm4* in rice cultivar PTB10

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ABSTRACT

Rice gall midge is considered as one of the most important insect pests of rice.Host plant resistance assumes a central role in pest management in order to alleviate crop loss as well as increase in crop production and productivity. Eleven gall midge resistant genes have been identified out of which eight genes have been mapped using molecular markers but their use in molecular breeding programs is still limited. The resistant gene, Gm4 identified from PTB10 is an ideal candidate for deployment in gene pyramiding. In the present study,in silico analysis was performed using'Rice GE'database to scan the fine mapped region of 0.141Mb on chromosome 8 between RM547 and RM22550. This region contains 412 transcriptional active genes out of which 49 candidate genes involved in host plant resistance mechanism were identified and primers were designed.Four oligo specific genes showing resistant linked allele suggesting their possible R gene mediated role in plant-pathogen interaction were validated with the F9, mapping population of TN1/PTB10. Further Marker Assisted selection using these candidate gene markers can be an important approach to develop stable resistance against rice gall midge.

Key words: *rice, gall midge, in silico analysis, candidate gene*

In Asia, more than 80% of the people live on rice, and their primary food security is entirely dependent on the volume of rice production.Significant constraints which limit rice production are insect pests and pathogens. Out of the 20 major insect pests,five insect pest *i.e.,* yellow stem borer (YSB), gall midge (GM), leaf folder (LF), brown planthopper (BPH), and white backed planthopper (WBPH), causes major damage in rice crop production (Katti G, 2013). Of these insects, Gall midge alonecausesa loss of more than US$700 million annually (Bentur *et al*., 2003). The Asian rice gall midge *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) is the most destructive pest of rice. The major symptom of damage caused by this insect is the formation of silvery white, tubular leaf sheath gall called silver shoot. The maggot, hatching from the egg, crawls down to the leaf sheath, feeds on the apical meristem and induces formation of gall, which renders the tiller sterile resulting in grain yield loss. Due to internal feeding habit of the pest, chemical control is less effective and environmentally unsafe. Thus the most feasible alternative to manage Gm infestation is the deployment of Gm resistant genes. Till date, 11 gall midge resistance genes have been identified out of which eight gall midge resistance genes (*Gm1 to Gm8)* have been tagged and mapped with different molecular markers. Several studies have reported the tagging and mapping of *Gm1* on chromosome 9, *Gm2, gm3*, *Gm 6(t*) and *Gm7* on chromosome 4, *Gm4* and *Gm8* on chromosome 8 and *Gm5* on chromosome 12 of rice (Biradar *et al*., 2004, Mohan *et al*., 1994, Katiyar *et al*., 2000, Katiyar *et al*., 2001, Sardesai *et al.,* 2002, Jain *et al*., 2004, Lima *et al*., 2007).However, none of the above single gene, is resistant to all the seven gall midge biotypes reported so far in India (Vijayalaxmi *et al*., 2006).

Hence, for development of durable resistance and to make the MAS breeding process faster candidate gene approach is an alternative strategy. Putative function of the gene of interest can be predicted based on the available DNA sequence information by using large genome databases. An in depth knowledge regarding resistance mechanism of plant and gallmidge

interaction is very much essential to find out the candidate gene, cloning and expression analysis.It has been reported that many of the gall midge resistance genes induce hypersensitive type of reactions in resistant genotypes and their reactions are classified as HR+ type. HR+ is a well characterized phenomenon in a plant pathogen interaction. A major dominant gene *Gm4* is known to exhibit HR+ response (Bentur and Kalode, 1996), which imparts resistance against four gall midge biotypes *i.e.* biotype 1, 2,3 and 4. The cultivar PTB 10 carrying the gene *Gm4*, is showing resistant reaction to gall midge infestation since last 15 years in the green house of CRRI (Sahu *et al*., 2004).This resistant gene *Gm4* in PTB10, has been mapped using F_9 RIL population between two microsatellite markers, RM547 and RM22550 placed at 1.9cM and 0.9cM away, and spanning 0.141 Mb in the short arm of chromosome 8 (Nanda *et al*., 2010).

Functional markers or perfect markers that are developed by referring the gene sequence obtained by map based cloning are generally considered as more precise markers. This is achieved by fine mapping the gene to the closest possible level followed by cloning to confirm the function. Then the sequence of the cloned gene or the allele is analyzed for the presence of functional nucleotide polymorphism and marker can be developed if the functional polymorphism exists corresponding to the presence and the absence of the particular allele-sequence. The function of the protein encoded by the cloned gene is determined by comparing with the amino acid sequence of known proteins. Several disease-resistance genes have been cloned from different plant species. Analyses of these genes reveal that, they share various similar sequences and common structural motifs, suggesting a common defense signal transduction pathway in different plant–pathogen interaction systems (Martin *et al.,* 2003).

Resistance-gene markers are a unique group within gene-targeted markers because they utilize specific features of genes involved in plant defense mechanisms. Consequently, it is expected that a large number of R genes plant^{-1} genome are able to confer resistance against a large spectrum of pathogens. Although different Rgenes respond to very different pathogens, they share several conserved regions (domains). Based on these domains, R genes have been classified into five groups: detoxifying enzymes (*e.g. Hm1* gene in maize), kinases (*e.g. Pto*gene in tomato), NBS/LRR proteins (the largest group, *e.g.* Arabidopsis *RPS2* and *RPM1* genes, tobacco *N* gene, tomato *Prf* gene, *fl ax L6* gene), extra cellular receptors (e.g. *Cf* gene of tomato) and receptor kinases (rice *Xa21* gene) (Song *et al.,*1995, Mago *et al*.,1998).

It has been observed that most of the above mentioned R genes possess conserved amino acid motifs of a nucleotide binding site domain and a hydrophobic domain with a consensus amino acid sequence GLPL (Gly-Leu-Pro-Leu) downstream of the NBS. This can be used to screen plant genomes for R genes and putative Rgenes (e.g., resistance gene analogs, RGAs), and to create molecular markers. With the identification of resistance gene analogues (RGAs), many investigators amplified similar regions from the genomes of diverse plant species. Some RGAs found to be mapped on the vicinity of either qualitative or quantitative resistance loci. These RGAs were thus candidate genes for new R-genes.

Recent advances in genomic databases have led to an explosion of data and a huge growth in both bioinformatics and biotechnology. With the available genomic sequences the challenge is to understand regulatory mechanisms of every single gene in the genome by computer analysis of the gene regulatory sequences and by integrating this data with biological knowledge of the gene. Sophisticated computational regulatory sequence analysis tools that employ powerful statistical and machine learning algorithms driven by the rich databases that collect known biological facts enable us to make profound *in silico* predictions and formulate experimentally testable hypotheses. Such *in silico* driven experiments can greatly speed up the process of our understanding of gene regulatory mechanisms and the identification of new target genes.

In the present study, with the knowledge of physical positions of *Gm4* gene linked markers in the rice genome, and available transcriptomics data, putative candidate genes were searched in the genomic regions encompassing 0.141 Mb regionon rice chromosome 8. The main objective was to find out all major disease resistance genes which are probably involved in Gall midge resistance and validate the propriety of the candidate genesfor the development of gene specific markers which can be effectively used

in MAS breeding programs for enhancing durability of resistant over time and space.

MATERIALS AND METHODS

The target region was identified on chromosome 8 for *in silico* analysis based on the flanking markers RM 22550 and RM547. The region on chromosome 8, spanning 0.9–1.9 cM was selected for this study supported by our own research findings (Fig 1A). Rice Functional Genomics Express Database (RiceGE, http://signal.salk.edu/RiceGE/RiceGE_Data_Source.html) was used to search the expressed genes surrounding the gall midge resistance gene present in the target regic-

Linkage map was done using gene specific primers OS08g09460, OS08g09650, OS08g09760, and OS08g14850. The resistance locus was found to be unanimous with the previously constructed map. The markers OS08go9460 is found to be highly linked to the resistance locus. With these new findings the resistant gene *Gm4* is flanked by the candidate gene marker OS08g09460 and OS08g 14850 at a distance of 0.4 cM and 1.0 cM respectively. This study magnifies the total distance of 2.9 cM present between the previous flanking markers RM 22550 and RM547.

The RICE GE was established by the Salk Institute Genome Analysis laboratory (SIGNAL) and

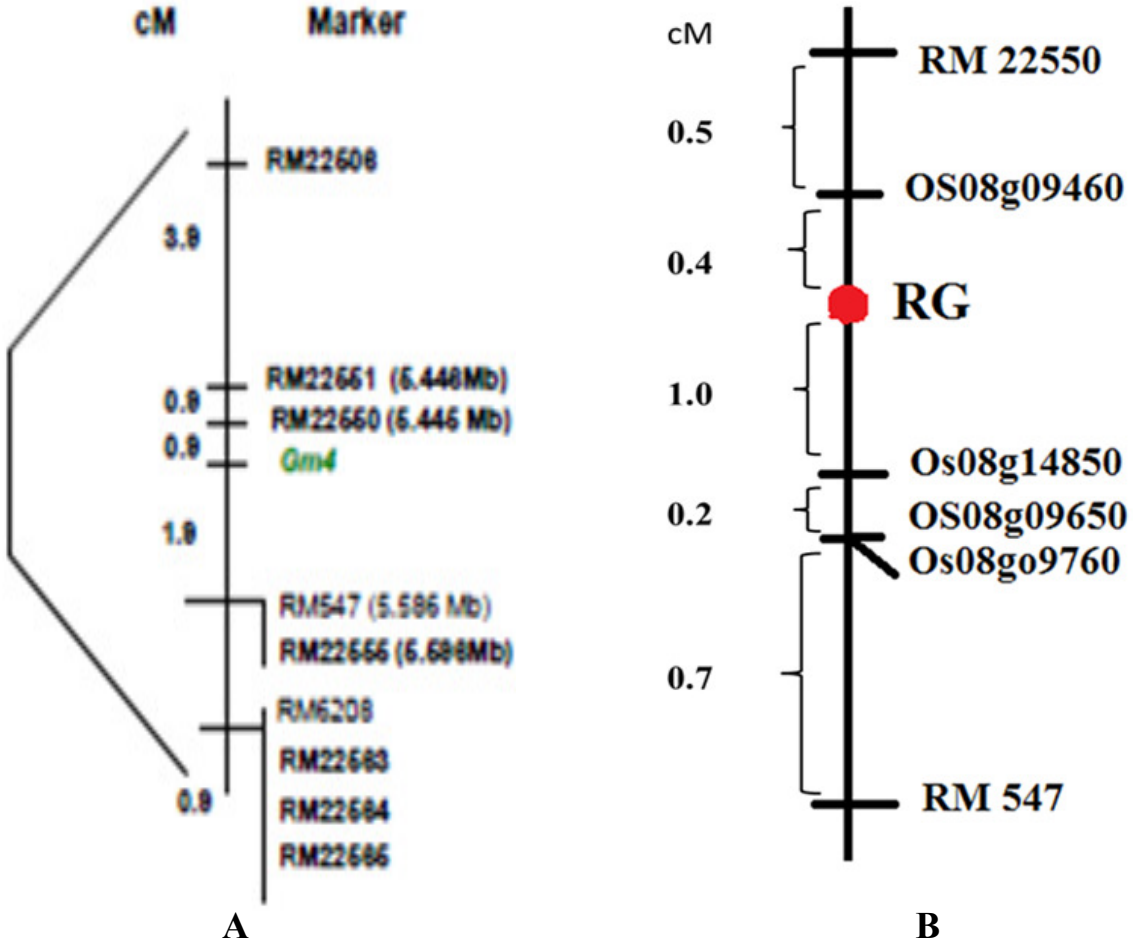

Fig. 1. High resolution mapping of gall midge resistance gene, Gm4 on short arm of rice chromosome 8. A: Map shows the position of linked microsatellite loci (bold) and *Gm4* identified using microsatellite primers. B:Map shows the position of linked oligospeciffic loci on right side of the figure. RG stands here for gall midge resistance gene *Gm4* in PTB10. Number on the left side of the map shows genetic distances between markers in centimorgan (cM). Numbers on the left side of respective maps shows the genetic distances (cM), while on the right side shows rice microsatellite loci. Genetic distances are not to the scale.

is one of the most comprehensive database and browser for rice functional genomics. With Rice GE, user could browse rice genome by gene name, CDNA, insertion name or chromosome region. Most impressively it could establish the relationship between gene and insertion, CDNA or other data which could not be done by any other browser. The genome browser incorporates the IRGSP genome sequence assembled by TIGR corresponding to pseudomolecules/annotation version 4. The Database also integrates rice full length CDNAs from the KOME Database and community CDNAs well as IRGSP BAC/PAC; rice ESTs, Markers and long SAGE tags.

The Website also provides convenient tool kit for rice functional genomics (http://signal.salk.edu/riceisects.4html). On the browser window of Rice GE using database associated search tools the target region was scanned for the presence of 412 putative candidate genes along with its function.The downloaded sequences of all the genes identified were saved in FASTA format.

Forty nine putative candidate genes were selected for designing of primers using Primer 3 (http://www.bioinformaticsnl/cgi.bin/ptimer3plus.cgi) software package.The product size, melting temperature, the likelihood of primer–dimer formation between the two primers in the pair, primer melting temperatures, primer length (18-25bp) and GC content (50-70%) were critically considered for designing primers.

Genomic DNA of resistant parent PTB10, susceptible parent TN1, and 110 RILs of TN1/PTB10 were isolated in the seedling stage using the 2% CTAB method. All the DNAs were quantified for the exact ratio. Parental polymorphism analysis was performed to identify primers amplifying bands/markers polymorphic between the parents using the 49 gene specific primers designed. Specificity of designed primers was tested by PCR amplification of the above genes. The PCR amplification was performed in a 20ì l reaction mixture volume containing 30ng of DNA, 1X PCR buffer, 200ì m dNTPs, 5 pM of forward and reverse primers, 2mM of magnesium chloride, and 1 ì l of *Taq* (*Thermos aquaticus*) DNA polymerase enzyme. A single primer pair was included in each PCR reaction.The reaction mixture was mixed well, 18 ìl was distributed to each tubes. 2 ìl of DNA sample from each genotype was added to corresponding tube, mixed well, briefly centrifuged to collect drops from wall of tube. The PCR was performed in a thermal cycler (LARK Thermal cycler) using following temperatures : initial denaturation at 93°C for 3 min followed by 40 cycles of denaturation at 94°C for 40sec, annealing at 55-67°C (depending upon primer) for 1 min and extension at 72°C for 1 min and final extension at 72°C for 7 min.10 ìl amplified products with 2 ìl of 6x loading buffer (Bromophenol blue) were loaded on 1.5% agarose gel containing ethidium bromide (10mg/ml) using 1X TBE buffer at 80Volt (50mA). The gels were visualized under UV and photographed using a gel documentation system. (Fluor ChemTM5500, Alpha Innotech, USA). The size of amplified fragments was determined by using DNA molecular weight markers (MBI Fermentas, Lithuania).The size of the DNA band/allele and matching was done by using Alphaease software (Alphainnotech, USA). Individual band within lanes were assigned to a particular molecular weight comparing with the DNA molecular weight markers. Total number of bands within each lane and number of polymorphic bands were noted.

RESULT AND DISCUSSION

A total of 412 genes with 40 BAC clones were discovered inthe target region comprising of 4610498-9045064 bp on rice chromosome 8 (Table 1). These genes mostly belonged to different categories based on sequence motifs/domains such as NBS-LRR, disease resistance, LRR, serine/threonine protein kinase, receptor kinase, receptor like kinase, F-Box domain, B-box zinc finger, NB-ARC domain, Cupin domain, retrotransposon, ATP binding protein, CRS2-associated factor 1, auxin efflux Carrier family protein, aconitatehydratase, ubiquitin-protein, starch synthase, bromodomain containing protein, zinc finger domain, hypothetical protein etc.These were unevenly distributed on the chromosome with a higher frequency of occurrence at certain physical positions. From putative candidate genes discovered in the study that possibly play role in gall midge resistance, we shortlisted a set of 49 genes for validation. Forty nine candidate genes selected for designing of gene specific primers belongs to 18 different BAC clones having 13 F-Box domain, 9 receptor like kinase, 11 germin/cupin like protein, 5 SHR5 receptor like protein, 3 disease resistance, 3 NBS-LRR protein and single gene each

Table 1. Forty-nine Candidate genes Selected for primer designing

Code	Position	BAC Clone	Description
Os08g08120	4610498-4610513	PO577G06	B-box zinc finger family protein, putative, expressed
Os08g08140	4630723-4631056	PO577G06	receptor-like kinase, putative, expressed
Os08g08230	4708277-4708675	P0455A11	kinesin-like protein, identical, putative, expressed
Os08g08920	5185878-5185998	P0020B10	Cupin domain containing protein
Os08g08960	5207437-5207560	B1099H05	Cupin domain containing protein, expressed
Os08g08970	5221278-5221398	P0610E02	Cupin domain containing protein, expressed
Os08g08980	5227883-5228003	P0610E02	cupin domain containing protein, expressed
Os08g08990	5232867-5232984	P0610E02	Cupin domain containing protein, expressed
Os08g09000	5238075-5238195	P0610E02	Cupin domain containing protein, expressed
Os08g09010	5241601-5241721	P0610E02	Cupin domain containing protein, expressed
Os08g09020	5247793-5247910	P0610E02	Cupin domain containing protein, expressed
Os08g09040	5253367-5253484	B1099H05	Cupin domain containing protein, expressed
Os08g09060	5259267-5259384	B1099H05	Cupin domain containing protein, expressed
Os08g09080	5263320-5263437	B1099H05	Cupin domain containing protein, expressed
Os08g09110	5273867-5276459	B1099H05	NB-ARC domain containing protein, expressed
Os08g09380	5440422-5440728	OSJNBa0056O06	F-box domain containing protein, expressed
Os08g09410	5451023-5452034	OSJNBa0056O06	F-box domain containing protein
Os08g09420	5456439-5457186	OSJNBa0056O06	F-box domain containing protein, expressed
Os08g09430	5465377-5468307	OSJNBa0056O06	disease resistance protein RPM1, putative
Os08g09460	5478729-5479482	OSJNBa0056O06	OsFBX267 - F-box domain containing protein
Os08g09650	5575470-5575800	P0035F08	OsFBX269 - F-box domain containing protein
Os08g09700	5601685-5601770	P0035F08	OsFBX270 - F-box domain containing protein, expressed
Os08g09710	5608492-5609595	P0035F08	OsFBX271 - F-box domain containing protein, expressed
Os08g09720	5617783-5618955	P0035F08	OsFBX272 - F-box domain containing protein, expressed
Os08g09730	5622925-5623980	P0035F08	OsFBX273 - F-box domain containing protein, expressed
Os08g09750	5632573-5633742	P0035F08	F-box domain containing protein, expressed
Os08g09760	5639020-5640168	P0035F08	F-box domain containing protein, expressed
Os08g09920	5727249-5728589	OSJNBb0094P23	F-box domain containing protein, expressed
Os08g10030	5810565-5810696	P0556A11	Nucleic acid binding protein
Os08g10260	5963413-5965600	OSJNBa0073I05	NBS-LRR disease resistance protein
Os08g10290	5979966-5980092	OSJNBa0073I05	SHR5 Receptor like kinase
Os08g10300	5997252-5997402	OJ1014_H11	SHR5 Receptor like kinase
Os08g10310	6014821-6014929	OJ1014_H11	SHR5 Receptor like kinase
Os08g10320	6031322-6031421	OJ1014_H11	SHR5 Receptor like kinase
Os08g10330	6039609-6039717	OJ1014_H11	SHR5 Receptor like kinase
Os08g10340	6054742-6056237	OJ1014_H11	OsFBX278 - F-box domain containing protein, expressed
Os08g10430	6122031-6122266	P0486F07	NBS-LRR disease resistance protein
Os08g10440	6137061-6137878	P0486F07	NBS-LRR disease resistance protein
Os08g10580	6221545-6222243	OJ1734_E04	Methyl CPG binding Domain Containing Protein
Os08g12680	7482311-7482919	OJ1119_E09	Zinc Finger Domain,LSD1
Os08g12740	7539414-7539449	OJ1006_H01	disease resistance protein RGA3
Os08g12750	7548307-7549220	OJ1006_H01	Serine/threonine Protein Kinase
Os08g13440	7988296-7988783	P0461A06	Cupin domain containing protein, expressed
Os08g14800	8893643-8894411	OJ1033_B09	disease resistance protein RPM1
Os08g14850	8933230-8933797	OSJNBa0049G15	disease resistance protein putative
Os08g14940	8995491-8995926	OSJNBa0049G15	Receptor like protein kinase precursors
Os08g14950	9010613-9013316	OSJNBa0049G15	Leucine rich repeat receptor protein kinase
Os08g14960	9019499-9020971	OSJNBa0049G15	Receptor like protein kinase precursors
Os08g14990	9033451-9036136	OSJNBa0049G15	Receptor like protein kinase precursors

of B-box zinc finger, NB-ARC domain, LRR domain, Methyl CPG domain, Nucleic acid binding protein which are considered to be the major candidate genes (Table 2) (Fig 2).

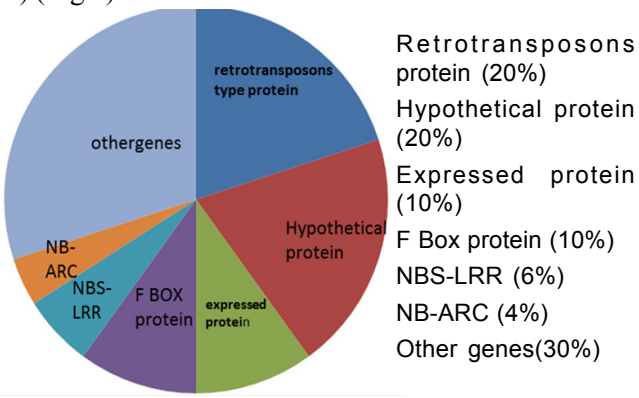

Fig. 2. Pie chart showing different genes underlying target region

Among the 40 BAC clones searched, eight, BAC clones consists of more than 20 genes. In some of the BAC clones, possible candidate genes like NBS-LRR,Cupin domain containing protein, NB-ARC domain containing protein, expressed, F-box domain containing protein, expressed, Zinc Finger Domain, LSD1, disease resistance protein RGA3,F Box Domain, Serine/threonine Protein Kinase, disease resistance protein RPM1, disease resistance protein putative, receptor like protein kinase precursors, Leucine rich repeat receptor protein kinase were identified which could probably have a role in rice gall midge interactions. Highest number of genes were present in the BAC clone OSJNBa0056006, which contains 32 diverse kind of genes like phosphorribosylamine-glycine ligase, putative, expressed (2), F-box domain containing protein (6), starch synthase III, putative, expressed (2),

Table 2. Polymorphic oligos with their specific function

Candidate gene	BAC Clone	Description	Function
Os08g08980	P0610E02	Cupin domain containing protein	Involves in regulation of seed germination and early seedling development.
Os08g08990	P0610E02	Cupin domain containing protein, expressed	Involves in plant growth and development. Although the majority of enzymatic Cupins contain iron as an active site metal, other members contain either copper, zinc, cobalt, nickel or manganese ions as a cofactor, with each cofactor allowing a different type of chemistry to occur within the conserved tertiary structure.
Os08g09110	B1099H05	NB-ARC domain containing protein, expressed	Known as class of defense genes it is the Cterminal part of NBS referred to as ARC sub domain conserved in plant NBS-LRR proteins.
Os08g09410	OSJNBa0056O06	F-box domain containing protein	F-box protein plays a crucial role in plant growth, development and innate immunity. F-box proteins exert controls over stability of proteins and regulate the mechanisms for a wide-range of cellular processes.
Os08g09420	OSJNBa0056O06	F-box domain containing protein,	
Os08g09460	OSJNBa0056O06	F-box domain containing protein	
Os08g09650	P0035F08	F-box domain containingprotein	
Os08g10440	P0486F07	NBS-LRR diseaseresistance protein	Generally thought to be involved in the interaction with avirulence (AVR) proteins and to be the major determinant of resistance specificity.
Os08g14800	OJ1033_B09	disease resistance protein RPM1	Involved in disease resistance
Os08g14850	OSJNBa0049G15	disease resistance protein putative	Involved in disease resistance
Os08g14990	OSJNBa0049G15	Receptor like protein kinase precursors	RLK genes expressed during seed development are also regulated by abiotic stresses (cold, salt, or drought) or hormones, indicating that RLKs may take part in the stress-related signaling pathways such as dehydration of endosperm
Os08g09760	P0035F08	F-box domain containing protein, expressed	

autophagy-related protein, putative, expressed (3), glyoxalase family protein, putative, expressed (3), tRNA synthetase, putative (1), pentatricopeptide, putative, expressed (1), hypothetical protein(5), expressed protein(3), gar2, putative,expressed(1), bromo domain containing protein, expressed(2), Polyprenylsynthetase, putative, expressed(1), disease resistance protein RPM1, putative(1),

Conserved hypothetical protein (1). Starch-synthase III (SSIII)gene is one of the enzymes involved in plants starch synthesis. Similarly autophagy related proteins are thought to one of the main mechanisms responsible for the degradation and remobilization of macromolecules and they play an important role in PCD (Plant Cell Death) in senescing tissue. All of the genes except the hypothetical proteins which were excluded in this study have specific role in plant growth and development and several studies have reported the major function of these genes not only in rice, but also in other crops like maize, sugarcane, potato, Arabidopsis (Table 2). Chandel *et al.,* (2010) suggested most of the RGAs were found in small clusters of 2-4 RGA percluster. Out of 11 RGAs, 5 RGAs belonged to receptor like kinase class, 5 belonged to LRR class including one NBS-LRR disease resistance gene and the remaining one was of serine/threonine protein kinase type for the region encompassing *Gm4* gene. In our study we identified 49 candidate genes relating to gall midge resistance covering 0.141Mb region of *Gm4* gene.

Out of the 49 gene specific primer pairs developed twelve primers specific to gene locus, Os08g08980, Os08g08990, Os08g09110, Os08g9410, Os08g9420, Os08g09460, Os08g09650, Os08g10440, Os08g14800, Os08g14850, Os08g14990 and Os08g09760 amplified polymorphic bands/ fragments between the susceptible parent TN1 and resistant parent PTB10 (Fig. 3). Among the 12 primer pairs, five primers (Os08g9410, Os08g9420, Os08g09460, Os08g09650, Os08g09760) have F box domain containing protein, two primers (Os08g14800, Os08g14850) have disease resistance RPM1 protein, 2 primers (Os08g08980, Os08g08990) have cupin like protein. Primer Os08g09110 has a single NB-ARC domain containing protein and Os08g10440 has only NBS-LLR domain.A single receptor like protein kinase was found in Os08g14990.Yasala *et al*;(2012) reported genes

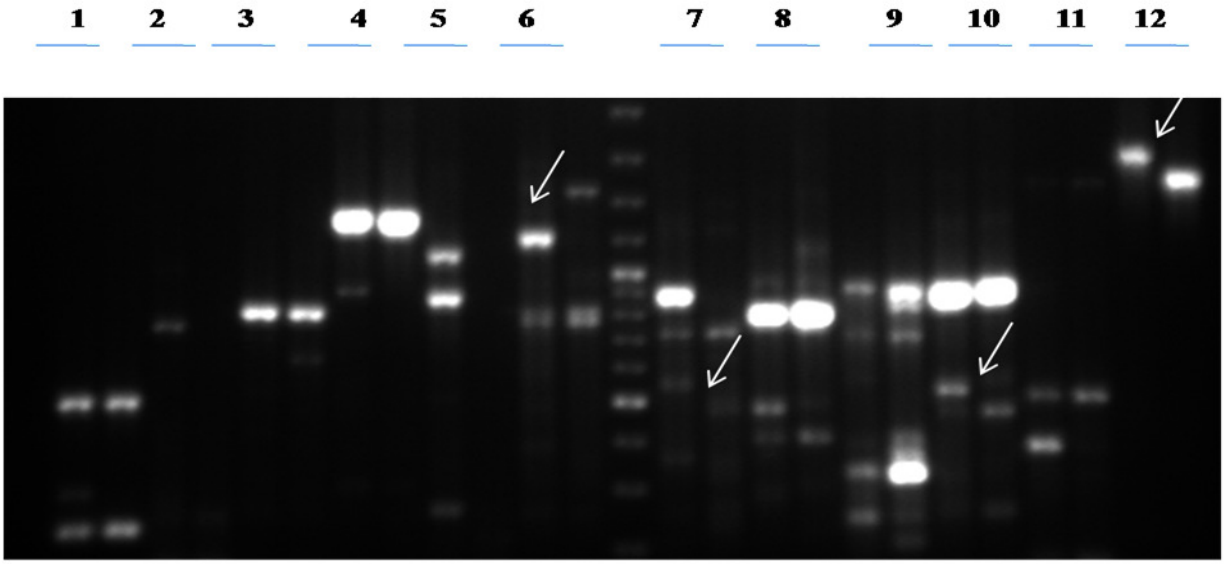

Fig. 3. Parental polymorphism analysis with genes specific primers

M = Molecular weight marker (100bp DNA ladder)
Pr = Resistant parent (PTB10)
Ps = Susceptible parent (TN1)
Primer numbers are indicated on the tope of the lanes as follows :
1 : OS 08g08980, 2 : OS 08g08990, 3 : OS 08g09110, 4 : Os 08g09410, 5 : OS 08g09420, 6 : OS 08g09460, M, 7: OS08g09650, 8 : OS 08g010440, 9 : OS 08g14800, 10 : OS 08g014850, 11 : OS08gO14990, 12 : OS08g09760

encoding F-box family protein were observed in high copy numberof 3 in *Gm2, Gm 2, Gm3, Gm 6, Gm7*, 25 number in *Gm4*and nine numbers in *Gm11* regions suggesting their involvement in the HR+ mediated gall midge resistance in rice.We found more number of polymorphic genesencoding F-Box domain protein, in comparison with other proteins. This necessitated to find out the exact role of F Box protein in rice-gall midge interaction. Though these proteins are part of ubiquitin-proteasome pathway which play role in ubiquitination of target proteins for their degradation, still our findings whichsupported with several similar findings, suggests its possible role in rice –gall midge interaction. In the present study co segregation analysis was performed with 110, F9 RILs of TN1/PTB10 population in order to identify and validate the candidate genes for gall midge resistance present in rice cultivar, PTB10 through *in silico* analysis. In co segregation analysis it was found that resistantspecific alleles of 1200bp,550 bp,520bp, 2000bp were amplified in all the resistant lines along with PTB10 where as susceptible specific alleles of 1520 bp, 470bp, 480bp, 1800bp were amplified along with TN1 in the primers OS08g09460, OS08g09650, OS08g14850 and OS08g09760 respectively (Fig. 3 and 4) (Fig 1 B). Rest two primers showed diversified banding pattern which was not suitable for further analysis. Validation of genes is important in order to identify and determine their specific functions. Himabindu (2009) amplified the putative candidate gene for Gm4 encoding LRR containing protein from resistant variety, Abaya (carrying Gm4) and susceptible variety, B95-1. Whereas, in this study resistant variety, PTB10 contains F-box protein putative candidate gene for *Gm4*.

In silico analysis provided the basis for the development of gene specific primers. The candidate genes identified (NBS-LLR, F-Box, disease resistance RPM1, NB-ARC) and the primers designed from among the putative candidate genes will help in confirming the involvement of the genes in gall midge resistance as well as for effective use in MAS breeding programmes. These results further enable us to associate functional genes and their interaction as an aid in understanding the elusive biological networks interaction involved in between the genes and in narrowing down to likely candidate genes for the gall midge resistance gene in chromosome 8.

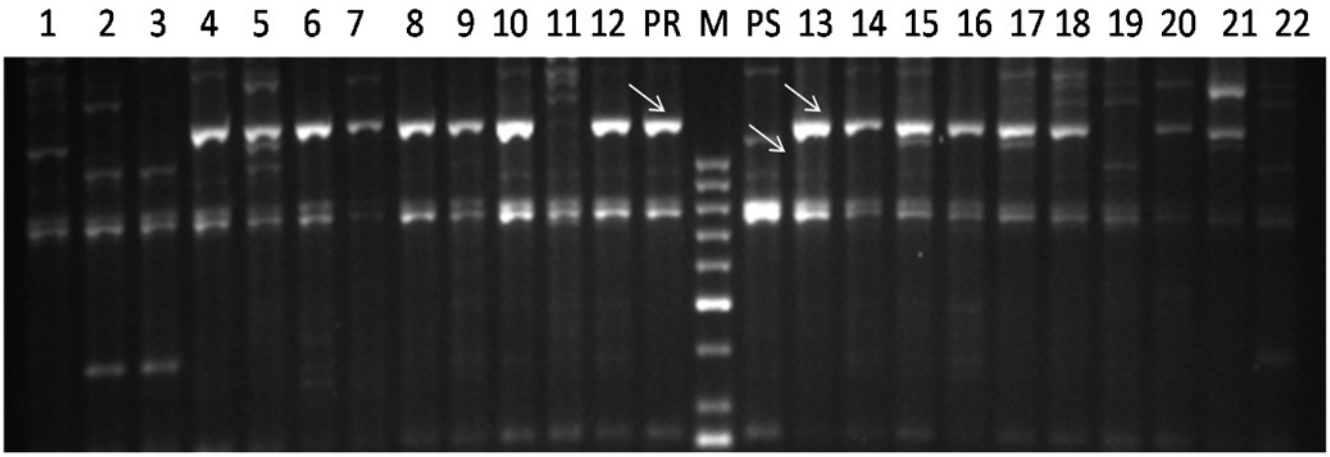

Fig. 4. Genotyping of F8 lines with gene specific primer, OS08g09460

M = Molecular weight marker (100bp DNA ladder)
Pr = Resistant parent (PTB10)
Ps = Susceptible parent (TN1)

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