The Ecoscan 10(1&2): 349-357, 2016 (Supplement on Rice)
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

MOLECULAR SCREENING OF RICE GENOTYPES USING LINKED MARKERS FOR GALL MIDGE RESISTANCE GENE, *Gm4*

SOUBHAGYALAXMI MOHAPATRA*, L. BEHERA, M. JENA, S. K. PRADHAN, S. K. MOHANTY AND S. C. SAHU
Pest genomics laboratory, Crop protection division,
National Rice Research Institute, Cuttack 753 006, Odisha
e-mail: slmohapatra2009@gmail.com

INTRODUCTION

Rice is the most important crop providing food for more than one-third of the world population. It is essentially a crop of warm humid environment conducive for the survival and multiplication of various insect pests. Due to its wide cultivation across several ecosystems, it invites numerous biotic stresses in the form of viruses, bacteria, fungi, parasites, insects, pathogen and weeds. Of these, insect pests alone cause more than 25% yield loss (Dhaliwal *et al*., 2010). Out of the 20 major insect pests, five insect pest *i.e.*, yellow stem borer (YSB), gall midge (GM), leaf folder (LF), brown plant hopper (BPH) and white backed plant hopper (WBPH) cause major damage in rice crop production (Katti, 2013). The Asian rice gall midge is ranked as the third most important insect pest which causes an annual yield loss of about 477,000 tons of grain and worth of US$80 million in eastern and southern India (Krishnaiah, 2004). Breeding and cultivation of resistant varieties is one of the best logical approaches to overcome gall midge problem. Many resistant varieties have been developed utilizing the resistant donors of gall midge and cultivated extensively. However, the extensive use of resistant varieties exerted high selection pressure on insect, and the lifespan of resistant varieties became short. This resulted development of new virulent gall midge biotypes with breakdown of resistance. So far, seven biotypes of gall midge have been reported in rice (Vijaya Lakshmi *et al*., 2006).

Development and use of molecular markers has played an increasing role in rice breeding and genetics during last few decades. The molecular markers that are tightly linked to the gene of interest have improved the efficiency of conventional plant breeding (Fraiture *et al*., 2016). The breeding has now become much more hassle free. Molecular markers that linked to the trait of interest would therefore provide a superior selection screen to assist in transferring resistance into improved cultivars. Among all the available markers, microsatellite markers are found to be the best since they are abundant, codominant, cost effective and interspersed throughout the genome (Vhora *et al*., 2013).

Till date, 11 gall midge resistance genes have been identified in rice (*Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9, Gm10* and *Gm11*). Eight genes *(Gm1, Gm2, gm3, Gm4, Gm6, Gm7, Gm8* and *Gm11*) have been tagged with different molecular markers and fine mapped on different chromosomes of rice. One gene (*Gm5*) has been tagged with molecular markers but not yet mapped (Bentur *et al*., 2016). *Gm1* is resistant to biotypes 1, 3, 5 and 6 while *Gm2* is resistant to biotype 1, 2 and 5. The recessive gene *gm3* imparts resistant to biotype 1, 2, 3, 4 and 7. Seven dominant genes, *Gm1, Gm2, Gm5, Gm6, Gm7, Gm9* and *Gm10* found to lack resistance against biotype 4. One of the important dominant gall midge resistance gene, *Gm4* has wide range of resistance containing F-box family protein, NBS LRR regions suggesting their involvement in the HR+ mediated gall midge resistance in rice (Mohapatra *et al*., 2014). The importance of the dominant gene *Gm4* has also been well explained by *insilico* analysis (Yasala *et al*., 2012). Previously, *Gm4* gene in PTB10 was found to impart resistance against biotype 1, 2, 3 and 4 and it was consistently resistant for 15 years against biotype 2 populations at NRRI, Cuttack (Sahu *et al*., 2004). This gene has been mapped on the short arm

ABSTRACT

Forty-eight rice genotypes comprising eighteen donors and thirty high yielding varieties were evaluated against biotype 2 of gall midge under glass house conditions at seedling stage. Three genotypes exhibited immune reaction while seven genotypes showed the high level of resistance to gall midge infestation. Two genotypes were moderately resistant while two genotypes were moderately susceptible. Thirty-four genotypes showed the susceptible reaction. These 48 rice genotypes were amplified with *Gm4* resistance gene linked four markers RM22550, RM22551, RM547 and RM22555. Based on the amplification pattern, the resistance gene *Gm4* from the cultivar PTB10 or Abhaya can be pyramided with other gall midge resistance genes present in the seven donors, Kavya, Samridhi, Phalguna, Dukong I, Suraksha, Aganni and BG380-2 in the background of twenty susceptible high-yielding varieties, Swarna, Samba Mashuri, Daya, Gajapati, Surendra, Ramachandi, CRdhan 500, Pooja, Tapaswini, Satabdi, Kharabela, B-95-1, CRdhan 300, Kanchan, Durga, Reeta, Naveen, Mahanadi, Ranjit and Sarathi through MAS breeding programs for development of durable gall midge resistance against biotypes 1, 2, 3, 4, 5, 6 and 7.

KEY WORDS
Gall midge
molecular screening
phenotyping
rice

Received : 07.09.2015
Revised : 18.01.2016
Accepted : 07.04.2016

***Corresponding author**

of chromosome 8 between two microsatellite markers, RM547 and RM22555 on one side while two microsatellite markers, RM22550 and RM22551 on other side of the gene in the cultivar PTB10 (Nanda *et al*., 2010).

A vast majority of high-yielding rice varieties are prone to gall midge attack, but few of the cultivars and land races are immune to it (Bentur *et al*., 2016). High vulnerability of existing popular varieties to gall midge attack is the result of narrow genetic variability and less diversified parents used in breeding. Thus, a critical analysis at the genetic variability is a prerequisite for initiating any crop improvement program (Patel *et al*., 2014). Interestingly, more than 95% rice germplasm collections worldwide have never been utilized in breeding programs. The phenotype screening is prerequisite for confirmation of recipient parent as well as finding new source of resistance that can be used in gene pyramiding programs.

In the quest for novel source of resistance, phenotype and genotype screening of rice cultivars with the available resistance gene linked markers have always remained as a suitable alternative before conducting any MAS program. One hundred gall midge resistant rice genotypes were screened under field condition against gall midge biotype GMB4M at Warangal and GMB1 in greenhouse at DRR (Dutta *et al*., 2014). Previously, a similar kind of work has also been carried out for gall midge resistance gene *Gm2* and *Gm4* and also for BLB in order to check the marker efficiency which gives credence to investigations (Dissanayake *et al*., 2005; Shikari *et al*., 2013). Thus, there is need for identification of additional non-allelic resistance genes to improve the durability of resistance in high yielding varieties replacing those olds. Looking into the above facts, the present study was carried out with the objective to identify resistant rice genotypes through phenotype screening, and to identify suitable donors-HYVs combinations for introgressing *Gm4* resistance gene into HYVs through MAS breeding program.

MATERIALS AND METHODS

The plant materials consist of eighteen donors having different gall midge resistance genes and thirty high yielding rice varieties (Table 1). These genotypes were used to test their reaction to gall midge infestation and also to test the suitability of *Gm4* gene linked markers for effective MAS breeding programs (Table 2).

The screening of 48 rice genotypes was carried in the Division Plant Protection glasshouse of National Rice Research Institute, Cuttack during 2014-15 using the method described by Bentur and Kalode (1996). The Cuttack population of gall midge is considered as biotype 2. Insects were mass reared on susceptible variety, TN1. Seeds of test genotypes were sown in lines in the plastic trays containing 5-7 cm height of soil in two replicates. The susceptible (TN1) and resistant (PTB10) checks were sown in each tray. Twelve to fifteen days old seedlings were thinned out keeping 25 seedlings in each line and each tray was exposed to 30 females and 15 males of gall midge in insect proof cages. Reactions were recorded 21 days after infestation using Standard Evaluation System score developed by IRRI(Anonymous, 2002). The evaluation was considered authentic when all the susceptible control plants of TN1 had silver shoots and the resistant PTB10 had no silver shoot.

Genomic DNA was isolated from 3-4gm leaves of 2-3 weeks old rice plants following CTAB method with minor modification (Ahmadikhah, 2008). The quantity and quality of DNA was estimated using spectrophotometer and agarose gel electrophoresis using known concentration of Lambda DNA. The samples were diluted in T10E1 buffer to get final concentration of 20ng/μl and were stored at -20°C for further use in amplification.The markers used for PCR amplification were linked SSR markers RM22550, RM22551, RM547 and RM22555 for *Gm4* resistance gene (Nanda *et al*., 2010). The primer sequences these markers were downloaded from Gramene Database (http://www.gramene.org) and custom synthesized by Qiagen Operon Technologies, Almeda, California (Table 2). Amplification was carried out in a 20μl reaction mixture volume containing 30-40ng of genomic DNA, 1X PCR buffer {75 mM Tris-HCl (pH 9.0), 50mM KCl, 20 mM $(NH_4)_2SO_4$}, 200μM dNTP mix (MBI Fermantas, Lithuania, USA), 5 picomole of each of forward and reverse primers, 2 mM of $MgCl_2$ and 1U of Taq *(Thermus aquaticus)* DNA polymerase (Biotools, Spain). The PCR was performed in a thermal cycler (Lark Thermal Cycler) using following cycling parameters: initial denaturation at 94ºC for 3 min followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 55-67ºC (depending upon primer) for 1 min and extension at 72ºC for 1.5 min and final extension at 72ºC for 5 min. Five micro liters of loading buffer was added to each tube of PCR product, mixed well. Ten micro liters of amplified products were separated on 2.5% agarose gel containing ethidium bromide using 1X TBE buffer. The gels were visualized under UV and photographed using a gel documentation system (Fluor Chem™ 5500, Alpha Innotech, USA) to detect polymorphism. The size of the DNA band/allele and matching was done by using Alphaease software (Alpha Innotech, USA). Individual band within lanes were assigned to a particular molecular weight comparing with the DNA molecular weight markers. Amplified products were scored as presence of resistance allele as P (PTB10), susceptible allele as T (TN1) and other allele as OA.

RESULTS AND DISCUSSION

Rice gall midge is considered as one of the major constraints in rice production. The resistant variety grown continuously in a locality becomes susceptible. This may be due to a change in the virulence of the pest or gradual build-up of a virulent population. The effective management strategy is the development of varieties with multiple non-allelic resistance genes to safeguard against possible change in virulence of gall midge. Conventional breeding procedure has inherent difficulties for simultaneous incorporation of many genes. It is only possible through marker-assisted breeding.

Two donors PTB10 and Abhaya along with HYV Moti showed immune reaction with SES score of 0. Five donors Lalat, Siam29, RP2068-18-3-5, RP 23331-156-8, MadhuriL9, and two high yielding varieties Konark and Jalamani showed resistant reaction with SES score of 1 (Table 3). The susceptible check TN1 showed 100% susceptibility with silver shoot formation. Only two donors *i*.e. BG380-2 and Kavya showed moderately

Table 1: List of rice genotypes used in study

Sl. No.	Cultivars(gene)	Parentage	Ecosystem*	Duration	Salient features**
1	W1263 *(Gm1)*	Eswarakora /MTU15	RSL	135	R to GM
2	Kavya (*Gm1*)	Mahsuri /Surekha	IRME	135	Semi dwarf, grains: MS, HR to GM, Yield: 65-70 Q/ha
3	Lalat (*Gm1*)	Vikram/W 1263	IRME	125	R to GM,BPH,GLH
4	Samridhi (*Gm1*)	IR22/ W1263	RSL	120	R to GM
5	ARC6605(*Gm1*)	entry of Assam rice collection	RSL	120	R to GM
6	Phalguna (*Gm2*)	IR8/Siam29	RSL	145	R to GM, MR to blast and SB
7	Siam 29 (*Gm2*)	landrace from thailand	RSL	120	R to GM
8	RP 2068-18-3-5 (*gm3*)	Swarnadhan/velluthachera	RSL	120	R to GM
9	PTB10 (*Gm4*)	Breeding line from patambi	RSL	90	R to GM
10	Abhaya (*Gm4*)	CR-157-392/OR-57-21	Irrigated	125	Semi dwarf, grains: LS, resistant to BLB, Blast and GM
11	ARC5984(*Gm5*)	Land race	RSL	120	R to gall midge
12	Dukong1(*Gm6*)	Kangewen2/wanbozao	RSL	120	R to gall midge
13	Surakshya(*Gm11*)	Sasyasree/CR57MR1523	IRM	120	R to gall midge
14	RP 23331-156-8(*Gm7*)	Ratna/ARC10659	RSL	120-130	R to gall midge
15	Aganni(*Gm 8)*	Landrace	RSL	120	R to gall midge
16	INRC 3021(*Gm8)*	Landrace	RSL	125	R to gall midge
17	MadhuriL9(*Gm 9)*	A mutant line of parents jaya/Dubraj	RSL	125	R to gall midge
18	BG 380-2(*Gm10*)	BGF90/24/BB67	RSL	135	SB grain R to GM
19	Ketakijoha	Badshahbhog /Savitri	RSL	145	MSG, R to blast
20	Swarna	Vasishta/Mahsuri	RSL	140	R to BLB and tolerant to many diseases. Yield:40Q/ha
21	SwarnaSub1	Swarna*3/IR49830-7	RSL	140	MS, tolerant to many diseases and submergence
22	Moti	CR-151-79 / CR-1014	RSL	145	LS, R to Blast, RTV, GLH, GM. good cooking quality. Height (115-120cm), Yield: 40-45 Q/ha.
23	Daya	Kumar/CR 57-49	IRME	120-125	MS,, Dwarf (70 cm), MR to blast, SB and BLB, R to GM, BPH, GH.Yield:40Q/ha
24	Gayatri	Pankaj/Jagannath	RSL	155	SB, Semi dwarf tolerant to SB
25	Gajapati	OR136-3/IR13429-196-1-120	IRM	130	Semi dwarf, grains - MS, white, tolerant to BPH; Yield: 35-50 Q/ha. R to Blast, Sh. R,LF, BPH
26	Sabita	Land race	RSL	130	Tall (150-160 cm), grains: LS; Yield: 40 Q/ha
27	MTU1010	Krishnaveni /IR-64	IRM	120	Semi-dwarf (108 cm), grains: LS, white, R to blast & tolerant to BPH; Yield: 74 Q/ha
28	Surendra	OR 158-5/Rasi	IRM	135	Semi dwarf, grains – MB, white, Yield: 35-50 Q/ha
29	Ramachandi	IR17494-32-2-2-1/ Jagannath	RSL	155	Semi dwarf, grains – MB, white, photosensitive; Yield: 45-65 Q/ha
30	CRdhan 500	Ravana/Mashuri	DW	158-163	MS, Height(140-155cm),MR to leaf blast, neck blast, BS, *Gm-1* & 5, SB, WM; R to rice thrips, leaf folder. Yield: 5 t/ha.
31	Varshadhan	IR31342-8/IR31406-3//IR26940-3-3-	Lowland	155-160	LBG, Plant height –150 cm; MR to neck blast, BLB. Yield: 35-40Q/ha
32	Tapaswini	Jagannath /Mahsuri	IRM	135	MSG, tolerant to WBPH, BB, MS to LF & GM Yield: 55 Q/ha
33	Savitri	Pankaj /Jagannath	RSL	150-155	Semi-dwarf (110-120 cm), SBG, good milling recovery, tolerant to blast & Sh. B, Yield: 38 Q/ha
34	Konark	Lalat/OR 135-3-4	IRM	125	Semi dwarf, MSG, white, tolerant to BPH ; Yield: 35-50 Q/ha
35	Pooja	Vijaya /T 141	RSL	140-150	MSG, R to blast
36	Satabdi	CR 10-114/CR 10-115	IRM	112-115	Semi dwarf , LSG, R to Sh.B, BB & Sh.R ; Yield: 35-56 Q/ha
37	Kharabela	Daya / IR13240- 108-2-2-3	IRM	120-130	MSG, Blast, RTV, Sh.R, GM, SB
38	B-951	Samba Mashuri /4/SS1113	RSL	135-140	Yield:4.75-5.0 t/ha, R to BLB
39	CR dhan 300	NDR9370018/KDML105//PSBRC60	RSL	135-140	LS,Yield:5.4 Q /ha
40	Kanchan	Jajati / Mahsuri	SLL	155-160	Tall (120-150 cm), MSG, resistant to blast, BLB, MR to Sh. B, BPH, & R to GLH; Yield: 40 Q/ha.
41	Durga	Pankaj / CR 1014	SDW	155-160	MS , R to RTV
42	Reeta	Savitri / IR 44	RSL	145-150	Semi dwarf (110 cm), MSG, R to leaf blast, SB, LF; MR to neck blast, BS Sh. B, Sh. R tolerant to yellow stem, BPH& LF; mod. Tolerance to GLH, WBPH rice thrips. Yield: 5 4.0 Q/ha
43	Jalamani	Panikekoa/Ambika	DW	140	yield o: 4.6 t/ha
44	Naveen	Sattari/ Jaya	RME	115-130	Plant height –105 cm; MBG ,R to blast & MR. to Sh. B, and SB.; Yield: 50-60 Q/ha
45	Samba Mashuri	GEB24/ TN1/ Mahsuri	RSL	140-150	MS,Yield:4.75-5.0 t/ha
46	Ranjit	Pankaj / Mahsuri	RME	155-160	Semi dwarf (99 cm) with quality grain of short fine, tolerant to BLB and S to blast, SB & GM; Yield: 40 Q/ha.
47	Sarathi	T90/IR8//W1263	IRME	115	Semi dwarf medium bold R to BLB, Blast, GM, GLH, SB
48	TN1	DGWG/ TSAIYUANCHUNG	NA	120-125	Dwarf (70-75 cm), grains: short bold, MR to blast, susceptible to BLB, RTV, GM, SB and moderately susceptible to SB

resistant reaction (SES score 3) while Aganni and Sarathi showed moderately susceptible reaction (SES score 5). Eight donors and twenty-six high yielding varieties showed susceptible reaction with more than 21% seedling damage and SES score of 7 -9 (Table 4).

Out of thirty high yielding varieties, three varieties, Moti, Konark and Jalamani exhibited resistant reaction to gall midge biotype 2 with less than 5% seedling damage. Similar results were reported by Behera *et al*. (2004) who screened 111 rice genotypes at seedling stage. The donors PTB10, Abhaya and RP 2333 showed resistant reaction. Similar results were obtained by Behera *et al*. (2004) and Sumathi and Manickam(2013). However, Phalguna and ARC5984 showed susceptible reaction in contrast to resistant reaction (Behera *et*

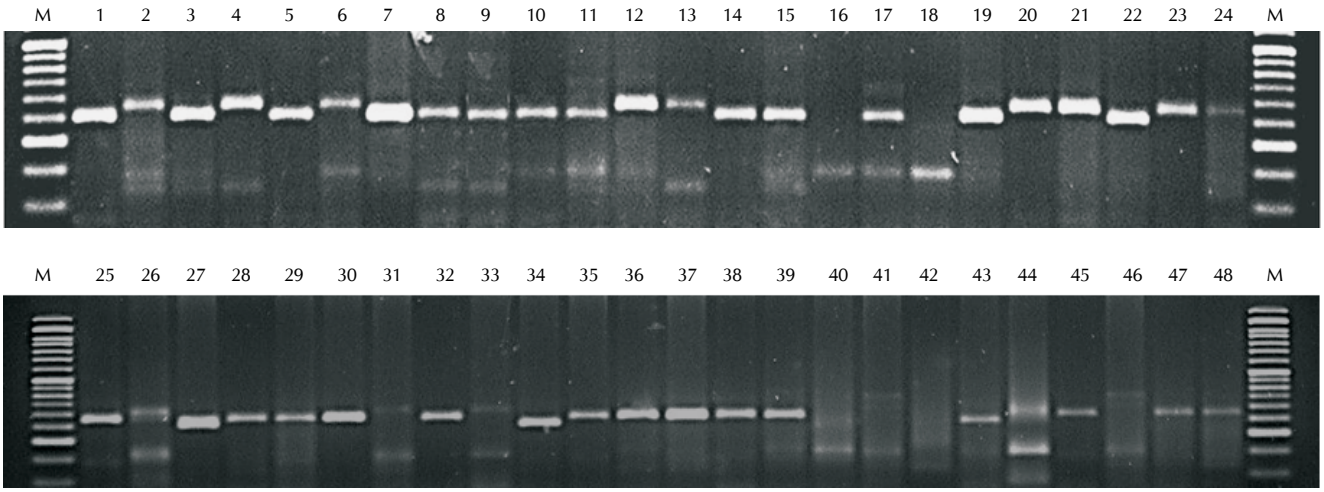

Figure 1: Amplification of genotypes with linked marker, RM 22550

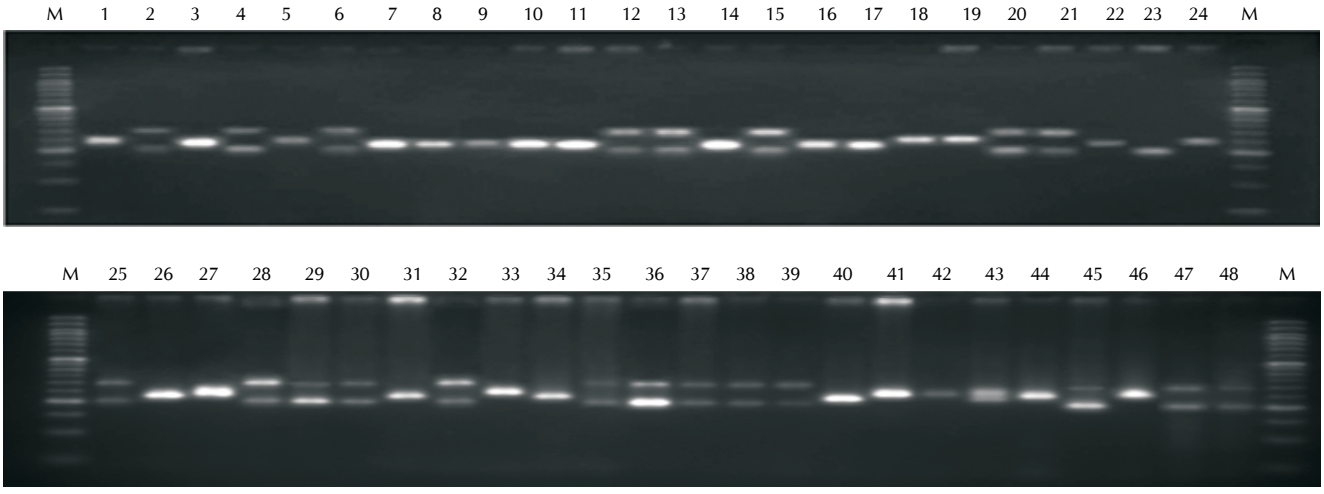

Figure 2: Amplification of genotypes with linked marker, RM 547

Table 2: Molecular markers used for screening of gall midge resistance gene *Gm4*

Gene/ chromosome	Marker locus		Sequence(5'-3')	Position (Mb)	Annealing temp in ºC
Gm4/Chr8	RM22550	(F)	CATTGCTTCTACTCACATGTCC	5.445	55
		(R)	GTTTAACCGATACAGGATGTGC		
	RM547	(F)	TAGGTTGGCAGACCTTTTCG	5.586	55
		(R)	GTCAAGATCATCCTCGTAGCG		
	RM22551	(F)	CTTCGATCTCCTCGTCCTCTTCC	5.446	55
		(R)	GAGCATGAGATGATGCATGACG		
	RM22555	(F)	GAGTTAGGGATCATCGAGCAAGG	5.596	55
		(R)	ATCGTGACGGTTAGATAGCAAGC		

al., 2004). Sumathi and Manickam(2013) screened 17 entries against gall midge biotype 3 in the Tirur district of Tamil Nadu .Six entries *viz*., ARC5984, Phalguna, Madhuri L 9, RP 2068-18-3-5, Abhaya and Aganni were found highly susceptible.

In case of molecular screening, amplification patterns of four *Gm4* resistance gene linked markers were studied in 18 gall midge donors and 30 high yielding varieties for their suitability in MAS breeding programs and identification of new donors. All the four linked markers RM22550, RM547, RM22551 and RM22555 amplified resistance specific alleles/bands of

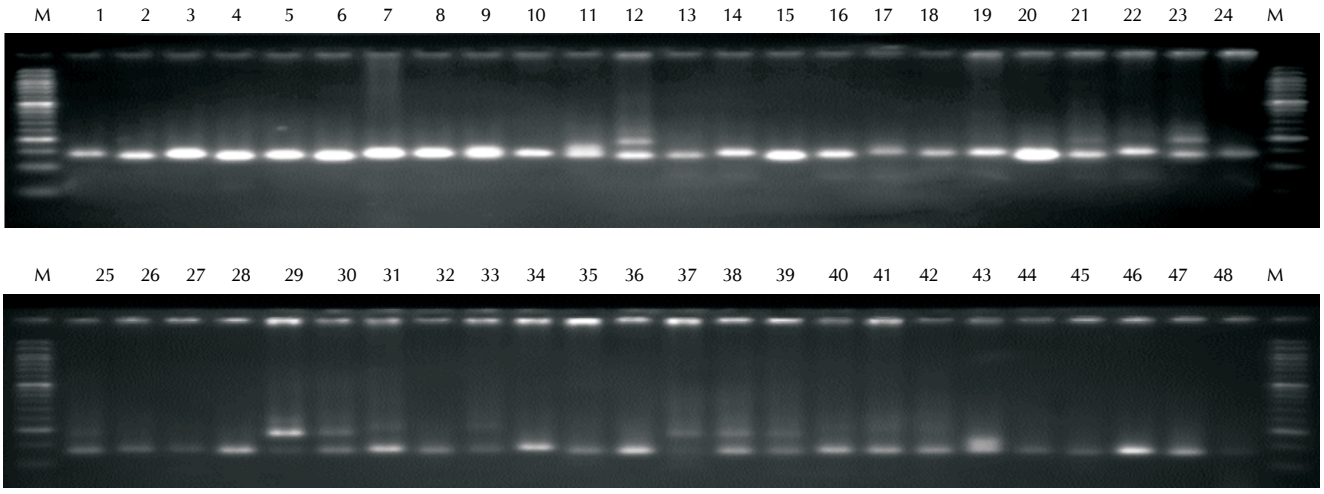

Figure 3 : Amplification of genotypes with linked marker, RM 22551.

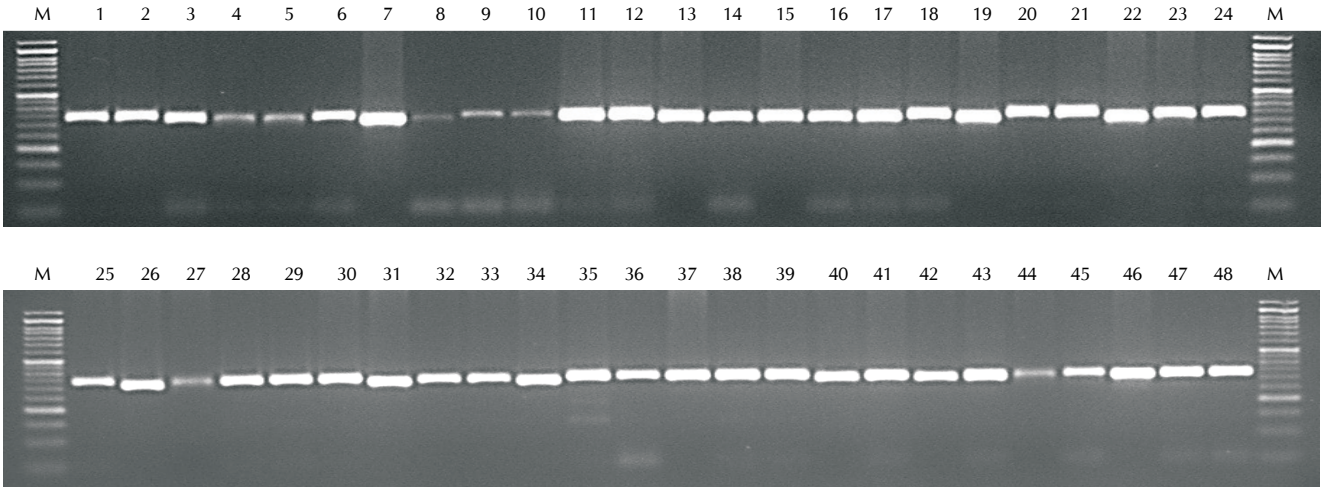

Figure 4: Amplification of genotypes with linked marker, RM 22555.

Fig.1-4: Amplification of genotypes with *Gm4* gene linked markers. Numbers on the top of the well represents the genotype in the similar order mentioned in the Table 1. M = Molecular weight marker in 50 bp.

260bp, 225bp, 140bp and 320bp, respectively in resistant donors PTB10 and Abhaya containing gall midge resistance gene *Gm4* Table 5. The susceptible alleles of 280bp, 203bp-298bp, 130bp and 330bp, respectively were amplified in susceptible check TN1 (Fig. 1-4). Similar kind of allele/banding pattern was obtained in these genotypes by Nanda *et al*. (2010) and Mohapatra *et al*. (2014). Resistance-linked marker alleles amplified by these four linked markers were present in seven donors Lalat (*Gm1*), Siam29 (*Gm2*), ARC6605 (*Gm2*), RP2068-18-3-5(*gm3*), ARC5984 (*Gm5*) and RP2333-1-156-8(*Gm7*), MadhuriL9(*Gm9*) while susceptible linked alleles were amplified in Kavya (*Gm1*) and Phalguna (*Gm2*). Similarly, two resistant HYVs Moti and Konark showed amplification of resistant specific alleles at four marker loci indicating that *Gm4* gene might be present in these two genotypes. Both *Gm2* and *Gm4* genes provide resistance against biotype 2. Hence, further experiment is required to confirm whether resistance in these two high yielding varieties is due to *Gm4* or *Gm2* gene or both. Based on the amplification pattern, suitability of flanking linked marker combinations was explored in pyramiding *Gm4* gene from PTB10/Abhaya with other gall midge resistance genes in the background of high yielding varieties. Four flanking marker combinations RM22550-RM547, RM22550-RM22555, RM22551-RM547 and RM22551-RM22555 were identified (Table 6). The resistance specific alleles amplified by flanking markers RM22550 and RM547 were present in donors Lalat, ARC6605- Siam29, RP2068-18-3-5, ARC5984, RP23331-156-8 and Madhuri L9 carrying *Gm1, Gm2*, gm3, *gm5, Gm7* and *Gm9,* respectively. However, these resistance specific alleles were not amplified in donors Kavya(*Gm1*), Samridhi(*Gm1*), Phalguna(*Gm2*), Dukong1(*Gm6*), Surakshya (*Gm11*), and HYVs Swarna, Samba Mashuri, Daya, Gajapati, Surendra, Ramachandi, CRdhan500, Pooja, Tapaswini, Satabdi, Kharabela, B951, CRdhan300, Mahanadi and Sarathi.

Table 3: Reaction of rice genotypes to gall midge biotype 2 population

Sl No	Name of the genotypes	% of silver shoot	SESS core	Status*	Sl No	Name of genotypes	% of silver shoot	SESScore	Status*
1	W1263	30	7	S	25	Gajapati	84	9	HS
2	Kavya	10	3	MR	26	Sabita	92	9	HS
3	Lalat	2	1	R	27	MTU1010	84	9	HS
4	Samridhi	94	9	HS	28	Surendra	68	9	HS
5	ARC6605	96	9	HS	29	Ramachandi	96	9	HS
6	Phalguna	80	9	HS	30	CRdhan 500	88	9	HS
7	Siam 29	4	1	R	31	Varshadhan	92	9	HS
8	RP 2068-18-3-5	2	1	R	32	Tapaswini	100	9	HS
9	PTB10	0	0	HR	33	Savitri	76	9	HS
10	Abhaya	0	0	HR	34	Konark	1	1	R
11	ARC5984	92	9	HS	35	Pooja	70	9	HS
12	Dukong1	54	9	HS	36	Satabdi	92	9	HS
13	Surakshya	79	9	HS	37	Kharabela	62	9	HS
14	RP 23331-156-8	3	1	R	38	B-95-1	70	9	HS
15	Aganni	18.5	5	MS	39	CRdhan 300	92	9	HS
18	INRC 3021	96	9	HS	40	Kanchan	100	9	HS
17	MadhuriL9	5	1	R	41	Durga	90	9	HS
18	BG 380-2	10	3	MR	42	Reeta	88	9	HS
19	Ketakijoha	92	9	HS	43	Jalamani	4	1	R
20	Swarna	100	9	HS	44	Naveen	94	9	HS
21	SambaMashuri	100	9	HS	45	Mahanadi	92	9	HS
22	Moti	0	0	HR	46	Ranjit	32	7	S
23	Daya	78	9	HS	47	Sarathi	16	5	MS
24	Gayatri	76	9	HS	48	TN1	100	9	HS

* HR- highly resistant, R- Resistant, MR- Moderately resistant, MS- Moderately susceptible, S-Susceptible, HS- Highly susceptible

Table 4: Reaction summary of different rice genotypes to biotype 2 of gall midge under artificial infestation condition

Seedling damage (%)	SES Score	Donors/ HYVs	Name of Genotypes	Remark*
0	0	Donors	Abhaya, PTB10	Immune/HR
		HYVs	Moti	
>0-5	1	Donors	Lalat, Siam29, RP2068-18-3-5, RP23331-156-8, MadhuriL9	R
		HYVs	Konark, Jalamani	
>5-10	3	Donors	BG 380-2, Kavya	MR
		HYVs	-	
>10-20	5	Donors	Agani	MS
		HYVs	Sarathi	
>20-50	7	Donors	W1263	S
		HYVs	Ranjit	
>50	9	Donors	Samridhi,ARC6605, Phalguna, ARC5984, Dukong1, Surakshya, INRC3021	HS
		HYVs	Ketakijoha, Swarna, Samba Mashuri, Daya, Gayatri, Gajapati, Sabita, MTU1010, Surendra, Ramachandi, Varshadhan, CRDhan500, Reeta, Kanchan, Durga Tapaswini, Savitri, Pooja, Satabdi, Kharavela, B-95-1, CRD300, Naveen, Mahanadi and TN1	

*R/S = Resistance/Susceptible reaction; SS = Silver Shoot; Score = 0- highly resistant(HR) (0% SS); 1- Resistant (< 5% SS)(R); 3- Moderately resistant (6-10% SS)(MR); 5- Moderately susceptible(11-20% SS)(MS); 7- Susceptible (21-50% SS)(S); 9- Highly susceptible (> 50% SS)(HS) (IRRI, 2002)(Scoring for net house)

The second set of flanking markers RM22551 and RM547 amplified resistance specific alleles in donors Lalat, Siam29 -ARC6605, RP2068-18-3-5, ARC5984, RP23331-156-8, MadhuriL9 carrying the genes *Gm1, Gm2, gm3, Gm5,Gm7* and *Gm9,* respectively while susceptible alleles were amplified in ten HYVs *i.e.,* Swarna, Samba Mashuri, Daya, Pooja, Satabdi, Kharavela, B951, CRdhan300, Mahanadi, Sarathi and six donors, Kavya- Samridhi, Phalguna, Dukong1, Suraksha and Aganni containing the genes *Gm1, Gm2, Gm6, Gm11* and *Gm8,* respectively.

The resistance specific alleles of flanking marker RM 22551 and RM22555 loci amplified in donors Lalat(*Gm1*), Siam29(*Gm2*),ARC6605(*Gm2*), RP2068-18-3-5(*gm3*), ARC5984(*Gm5*), RP23331-156-8(*Gm7*) and MadhuriL9(*Gm9*) while susceptible alleles were amplified in donors Kavya(*Gm1*), Phalguna(*Gm2*) and BG380-2(*Gm10*). Similarly, resistance specific alleles of RM22550 and RM22555 flanking markers were amplified in donors Lalat (*Gm1*), ARC6605 (*Gm2*), Siam29 (*Gm2*), RP2068-18-3-5(*gm3*), ARC5984 (*gm5*) and RP23331-156-8(*Gm7*) while susceptible specific alleles in Kavya(*Gm1*)

Table 5: Amplification pattern of forty-eight genotypes with *Gm4* gene linked markers

Sl.No	Genotypes	Gall midge resistance gene(s)	Reaction to gall midgebio type 2*	Marker allele amplification**			
				RM22550 P-260bp T-280bp	RM2255 1P-140bp T-130bp	RM547P -225bpT -200,298bp	RM22555 P-320bp T-330bp
1	W1263	*Gm1*	S	P	P	OA	T
2	Kavya	*Gm1*	R	T	T	T	T
3	Lalat	*Gm1*	R	P	P	P	P
4	Samridhi	*Gm1*	S	T	T	T	P
5	ARC6605	*Gm2*	S	P	P	P	P
6	Phalguna	*Gm2*	S	T	T	T	T
7	Siam 29	*Gm2*	R	P	P	P	P
8	RP 2068-18-3-5	*gm3*	R	P	P	P	P
9	PTB10	*Gm4*	R	P	P	P	P
10	Abhaya	*Gm4*	R	P	P	P	P
11	ARC5984	*Gm5*	S	P	P,OA	P	P
12	Dukong1	*Gm6*	S	T	T,OA	T	P
13	Suraksha	*Gm11*	S	T	T	T	P
14	RP 23331-156-8	*Gm7*	R	P	P	P	P
15	Aganni	*Gm8*	S	P	T	T	P
16	INRC 3021	*Gm8*	S	OA	T	P	P
17	MadhuriL9	*Gm9*	R	P	P	P	P
18	BG 380-2	*Gm10*	R	OA	T	OA	T
19	Ketakijoha	None	S	P	T	OA	P
20	Swarna	None	S	T	T	T	T
21	Samba Mashuri	None	S	T	T	T	T
22	Moti	None	R	P	P	P	P
23	Daya	None	S	T	T,OA	T	OA
24	Gayatri	None	S	T	T	P	OA
25	Gajapati	None	S	T	P,OA	T	T
26	Sabita	None	S	OA	P	P	P
27	MTU1010	None	S	OA	P	OA	T
28	Surendra	None	S	T	P	T	T
29	Ramachandi	None	S	T	P,OA	T	T
30	CRdhan 500	None	S	T	P,OA	T	T
31	Varshadhan	None	S	T,OA	P,OA	P	P
32	Tapaswini	None	S	T	OA	T	T
33	Savitri	None	S	T,OA	OA	OA	T
34	Konark	None	R	P	P	P	P
35	Pooja	None	S	T	T	T	T
36	Satabdi	None	S	T	T	T	T
37	Kharabela	None	S	T	T,OA	T	T
38	B-95-1	None	S	T	T,OA	T	T
39	CRdhan 300	None	S	T	T,OA	T	T
40	Kanchan	None	S	P,OA	T	P	T
41	Durga	None	S	OA	T	OA	T
42	Reeta	None	S	OA	T	OA	T
43	Jalamani	None	R	P	T,OA	P,OA	T
44	Naveen	None	S	T	T	OA	T
45	Mahanadi	None	S	T	T	T	T
46	Ranjit	None	S	OA	T	OA	T
47	Sarathi	None	S	T	T	T	T
48	TN1	None	S	T	T	T	T

* R-Resistant, S-Susceptible, ** P- PTB10 allele, T-TN1 allele, OA-Other allele

and Phalguna(*Gm2*). These markers amplified polymorphic alleles between resistant genotypes PTB10 and Swarna, Samba Mashuri, Gajapati, Surendra, Ramachandi, CRdhan500, Tapaswini, Savitri, Pooja, Satabdi, Kharabela, B951, CRdhan300, Navin, Mahanadi and Sarathi.

The alleles of different size other than resistant and susceptible alleles were classified as other alleles (OA). RM547 amplified other alleles (OA) in two donors W1263, BG380-2, and eight HYVs Ketakijoha, MTU1010, Savitri, Durga, Reeta, Jalamani, Naveen and Ranjit . Similarly, other alleles were amplified by RM22550 and RM22551 loci in resistant donors BG380-2, INRC3021, ARC 5984 and Dukong1. Among the donors, out of 144 alleles, 62.5% alleles were found to be of resistant specific (PTB10 type), 33.3% alleles were of susceptible

Table 6: Suitability of flanking marker combination for introgression of resistance gene, *Gm4* into elite high yielding rice varieties

Donors	Flanking marker combination(RM)	Donors/HYVs(Recipient parents)
PTB10/Abhaya	RM22550,RM547	Donors:Kavya(*Gm1*), Samridhi(*Gm1*), Phalguna(*Gm2*), Dukong1(*Gm6*), Suraksha (*Gm11)*HYVs:Swarna, Samba Mashuri, Daya, Gajapati, Surendra, Ramachandi, CRdhan500, Pooja, Tapaswini, Satabdi, Kharabela, B951, CRdhan300, Mahanadi,Sarathi.
	RM22551,RM22555	Donors:Kavya(*Gm1*),Phalguna(*Gm2*), BG380-2(*Gm10*) HYVs : Swarna, Samba Mashuri, Pooja, Satabdi, Kharavela, B951, CRdhan300, Kanchan, Durga,Reeta, Jalamani, Naveen, Mahanadi, Ranjit, Sarathi
	RM22550,RM22555	Donors:Kavya(*Gm1*), Phalguna*(Gm2*) HYVs : Swarna, Samba Mashuri, Gajapati, Surendra, Ramachandi, CRdhan500, Tapaswini, Savitri, Pooja, Satabdi, Kharabela, B951, CRdhan300, Naveen, Mahanadi, Sarathi
	RM22551,RM547	Donors:Kavya(*Gm1*), Samridhi(*Gm1*), Phalguna(*Gm2*), Dukong1(*Gm6*), Surakshya(*Gm11*), Agani(*Gm8*):HYVsSwarna, Samba Mashuri, Daya, Pooja, Satabdi, Kharavela, B951, CRdhan300, Mahanadi , Sarathi

specific (TN1 type) and the rest of the alleles were considered as other alleles (4.16%). Most of genotypes were susceptible to gall midge and in general it is found to have abundant matches of alleles with the susceptible allele of TN1.

Our study further revealed that nine HYVs like Swarna, Samba Mahsuri, Pooja, Satabdi, Kharabela, B951, Sarathi, Mahanadi and CRdhan300 contained susceptible alleles for all the four linked markers. These varieties can be used as recipient parents for successful transfer of gall midge resistance gene *Gm4* from PTB10/Abhaya. Seven genotypes, Kavya, Samridhi, Phalguna, Dukong I, Suraraksha, Aganni and BG380-2 can be used as donors to pyramid other gall midge resistance genes with *Gm4* from PTB10/Abhaya in the background of twenty susceptible high yielding varieties, Swarna, Samba Mashuri, Daya, Gajapati, Surendra, Ramachandi, CRdhan 500, Pooja, Satabdi, Kharabela, B-95-1, CRdhan 300, Kanchan, Durga, Reeta, Naveen, Mahanadi, Ranjit, Tapaswini and Sarathi through MAS breeding programs for development of durable gall midge resistant varieties against biotypes1, 2, 3, 4, 5 ,6 and 7. We identified two HYVs Moti and Konark resistant to biotype 2 which can be directly cultivated by farmers in gall midge biotype2 endemic areas**.** Dash *et al*. (2004) reported many resistant and moderately resistant varieties like Heera, Kalinga-II, Neela, Tara, Khandagiri, Udaya, Daya, Gouri, Pratap, Shakti, Phalguna,Meher, Birupa, Bhanja and Samanta for medium lands, and Samalei, Manika and Urbashi for low lands to reduce gall midge damage considerably. Meher *et al*. (2009) reported that few genotypes from early group viz., Ananga, Annada, Kharavela and Shaktiman showed highly resistant reaction at both the levels of nitrogen with 0% silver shoot.

Some deviations were observed. Four genotypes ARC6605, ARC5984, INRC3021 and Sabita which showed resistant reaction in earlier reports (Behera *et al*., 2004), now showed susceptible reaction to gall midge biotype 2. The presence of resistance specific alleles in these donors suggested that *Gm4* gene may be unexpressed and silent. The evolution of plant resistance gene in genotypes is due to the result of unequal crossing over, gene conversion, and point mutation, which leads to genetic variability and the generation of new specificities. Genic and intergenic sequence repeats generated by duplication and transposon insertion that provide unequal crossing over and inter-locus gene conversion. Thus, the intergenic unequal crossing over has the potential to replace resistance gene in new structural contexts that may alter expression, in some genotypes where as intragenic mispairing generates chimeric genes that may encode novel functions in some genotypes.

REFERENCES

Ahmadikhah, A. 2008. A rapid mini-prep DNA extraction method in rice (*Oryza sativa* L.).*Af. J. Biotechnol***. 8(2):** 323-327.

Anonymous, 2002. Standard evaluation system for rice. International Rice Research Institute, Manila, p.56.

Behera, L., Sahu, S. C., Rajamani, S., Subudhi, H. N., Bose, L. K. and Singh, B. N. 2004. Screening of rice cultivars against rice gall midge (*Orseolia oryzae* Wood Mason) under glass house conditions. *Oryza* **41:** 61:63.

Bentur, J. S. and Kalode, M. B. 1996. Hypersensitive reaction and induced resistance in rice against Asian rice gall midge *Orseolia oryzae*. *Entomol. Exp. Appl*. **78:** 77-81.

Bentur, J. S., Rawat, N., Divya, D., Sinha, D. K., Agarrwal, R., Atray, I. and Nair, S. 2016. Rice–gall midge interactions: Battle for survival. *J. Insect. Physiol*. **84:** 40-49.

Dash, A. N. 2004. The rice gall midge problem in Orissa. In : New Approaches to Gall Midge Resistance in Rice ,Bennett.J, Bentur,J.S., Pasalu,I.C. and Krishnaiah,K (Eds.). Proc. the International Workshop, 22-24 November 1998, Hyderabad, India. Los Baños, Philippines. p.195.

Dhaliwal, G. S., Jindal,V. and Dhawan, A. K. 2010. Insect Pest Problems and Crop Losses: Changing Trends. *Ind. J. Ecol*. **37(1):** 1-7.

Dissanayake, D. M. K. X., Fernando, K. K. S. and Bandara, J. M. R. S. 2005. Molecular Screening of Selected Rice Varieties with Specific Markers for *Gm2* and *Gm4(t)* Gall Midge Resistant Genes. *Trop. Agril. Res.* **17:** 48-57

Dutta, S. S., Divya, D., Ch, V Durga Rani., Reddy, D., Visalakshmi, V., Cheralu, C., Singh, K. I. and Bentur, J. S. 2014. Characterization of gall midge resistant rice genotypes using resistance gene specific markers. *J. Exp. Bio. and Agril. Sc.* **2(4):** 439-446.

Fraiture, M. A., Roosens, N. H. C, Taverniers, I., Loose, M. D., Deforce, D. and Herman, P. 2016. Biotech rice: Current developments and future detection challenges in food and feed chain. *Trends in Food Science & Technology*. **52:** 66-79.

Katti, G. 2013. Biopesticides for Insect Pest Management in Rice – Present Status and Future Scope. *J. Rice Res.* **6(1):** 1-15.

Krishnaiah, K. 2004. Rice gall midge, *Orseolia oryzae* - an overview. In: New approaches to gall midge resistance in rice. Bennett. J,

Bentur,J. S, Pasalu I. C and Krishnaiah .K (Eds). Proc. the International Workshop, 22-24 November 1998, Hyderabad, India. Los Baños, Philippines. pp.1-5.

Mehar, J., Dani, R.C. and Subudhi, H. N. 2009. Field screening of improved rice genotypes against the Asian rice gall midge (*Orseolia oryzae* Wood-Mason). *Oryza* **46** : 48-52.

Mohapatra, S., Panda, R. S., Mohanty, S. K., Behera, L., Sahu, S. C. and Prakash, A. 2014. *In silico* analysis of gall midge resistance gene *Gm4* in rice cultivar PTB10. *Oryza* **51(1):** 34-42.

Nanda, A., Mohanty, S. K., Panda, R. S., Behera, L., Prakash, A. and Sahu S.C. 2010. Flanking microsatellite markers for breeding varieties against Asian rice gall midge. *Trop. Plant Biol.* **3:**219-226.

Patel, D., Patel, D. A., Chakraborty, S., Parmar, D. J. and Jadav, N. J. 2014. Molecular characterization of rice (*oryza sativa l.*) genotypes resistant to moisture stress. *The Bioscan.* **9(2):** 899-905.

Sahu, S. C., Bose, L. K. and Behera, L. 2004. Inheritance of resistance against three biotypes of the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason). *Oryza* **41:** 59-61.

Shikari, A. B., Khanna, A., Gopala Krishnan, S., Singh, U. D., Rathour, R., Tonapi, V., Sharma, T. R., Nagarajan, M. K., Prabhu, K. V. and Singh, A. K. 2013. Molecular analysis and phenotypic validation of blast resistance genes *Pita* and *Pita2* in landraces of rice (*Oryza sativa* L.). *Ind. J. Genet.* **73(2):** 131-141.

Sumathi, E. and Manickam, G. 2013. Field screening of rice accessions against rice gall midge (*Orseolia oryzae* Wood-Mason). *Crop Res.* **45** (**1, 2 & 3):** 54-58.

Vhora, Z., Trivedi, R., Chakraborty, S., Ravikiran, R. and Sasidharan, N. 2013. Molecular studies of aromatic and non-aromatic rice (*Oryza sativa* L.) genotypes for quality traits using microsatellite markers. *The Bioscan* **8:** 359-362.

Vijaya Lakshmi, P., Amudhan, S., Himabindu, K., Cheralu, C., Bentur, J.S. 2006. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. *Int. J. Trop. Insect Sci.* **26:** 207-211.

Yasala, A. K., Rawat, N., Sama, V. S. A. K., Himabindu, K., Sundaram, R. M. and Bentur, J. S. 2012. Analysis for gene content in rice genomic regions mapped for the gall midge resistance genes. *Plant Omics J.* **5:** 405-413.