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Possible Detection of the Rice Gall Midge (*Orseolia oryzae*) Resistance Genes in Rice Genotypes Using Relevant Markers

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Abstract

The Asian rice gall midge (*Orseolia oryzae*) poses a significant threat to rice (*Oryza sativa* L.) cultivation, causing substantial yield losses of 0.8 % of the total production, amounting to US $ 80 million annually. Use of resistant rice varieties offers an effective strategy for managing this pest, particularly through the incorporation of multiple resistance genes. This study utilized functional (*gm3*, *Gm4*, *Gm8)* or linked molecular markers (*Gm1*, *Gm2*, *Gm11*, and *gm12*) to identify resistance alleles associated with gall midge resistance genes in a collection of rice genotypes. The results revealed the presence of multiple resistance alleles in various combinations in several genotypes, like PTB 20, RP 6290-22, RP 6749-7-19, RP 6810-20 and Karma Mahsuri. However, it appears that presence of multiple alleles may not always confer absolute resistance to this pest as evident from the performance of these lines in multilocation evaluation trials. This would ensure better deployment of resistant genes ensuring location specific gall midge management.

Keywords: Rice, gall midge, multiple resistance, molecular markers

Introduction

Rice (*Oryza sativa* L.) is one of the three most important cereal crops and a staple food for more than half of the global population. It is grown under diverse environments such as flooded irrigated or rainfed paddies, or dry uplands (Singh *et al*., 2021). Nutritionally, its endosperm is rich in starch (70–80 %), protein (7–10 %), and lipids (around 1 %) making it a key dietary staple (Yang *et al.,* 2019). Among the major pests that limit the potential yield of the crop, the Asian rice gall midge [*Orseolia oryzae* (Wood-Mason)] is ranked third most important insect pest in India, following stem borers and planthoppers (Bentur *et al.,* 2016), causing an annual yield loss of

approximately 0.8 %, equating to US $ 80 million (Mathur and Krishnaiah, 2004). Its internal feeding behavior renders chemical control largely ineffective. The most effective management strategy is the use of resistant rice varieties (Katti, 2021). In India, though over 100 gall midge-resistant rice varieties have been developed for commercial cultivation, eight of these are in seed production chain for 9 to 21 years (Padmakumari *et al.,* 2024). Of late, since only *Gm1* and *Gm8* are found effective in most of the locations through virulence monitoring trials (IIRR 2018-2024), breeding lines with two to three gene combinations are developed in the background of Improved Samba Mahsuri (Abhilash *et al.,* 2017), Swarna (Shilpi *et al.,* 2020), Naveen (Perumalla *et al.,* 2021) etc.

Intensive research over the last two decades at the Indian Counsil of Agricultural Research– Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad and other centers in India have helped gain an insight into genetic and molecular bases of rice-gall midge interactions (Bentur *et al.,* 2016). To date, 12 major resistance genes (*Gm1–gm12*) have been identified, (Himabindu *et al.,* 2010; Leelagud *et al.,* 2020), of which two genes, *gm3* and *gm12*, are recessive. Two of these genes, *Gm1* and *Gm8*, express HR$^-$ type response without the hypersensitive response that is manifest with other genes (VijayKumar *et al.,* 2022). Ten of the genes have been tagged and mapped on different chromosomes (Nair *et al.,* 2011; Leelagud *et al.,* 2020). While four of the genes, *Gm2, gm3, Gm6*, *Gm7* are located on chromosome 4, two (*Gm4, Gm8*) on chromosome 8 and (*Gm11* and *Gm5*) on chromosome 12 (Zhou *et al.,* 2020); one each on chromosome 9 (*Gm1*), and 2 (*gm12*). Three of the gall midge resistance genes have been cloned and functionally characterized (Sama *et al.,* 2014; Divya *et al.,* 2015, 2018). These studies have identified and developed functional, or gene linked markers that have been used in marker assisted selection (MAS) for gene pyramiding to breed for durable gall midge resistance (Abhilash *et al.,* 2017).

This study has utilized specific molecular markers to identify resistance genes in a selected set of gall midge resistant breeding lines with varying levels of resistance to various populations of gall midge currently prevailing in India (ICAR-IIRR, 2024).

Materials and Methods

Plant Material

Molecular analysis was performed on rice genotypes selected from the collection of the ICAR-Indian Institute of Rice Research, Hyderabad on the basis of the phenotyping carried out under multilocation testing in gall midge screening trial of **All India Coordinated Research Project on Rice (AICRPR)** wherein the plants with 0-10 % plant damage or less than or equal to 1 % silver shoot are scored as resistant and >10 % plant damage as susceptible. (ICAR-IIRR, 2024). The detailed information regarding these genotypes is provided in **Table 1.**

DNA extraction and PCR

The rice genotypes were grown in pots in the greenhouse at the Agri Biotech Foundation, Hyderabad, during the *kharif* season of 2024. DNA was extracted from rice seedlings using the modified CTAB (Cetyl Trimethyl Ammonium Bromide) method described by Saghai-Maroof *et al*., (1984). Reported functional/gene linked markers, detailed in **Table 2,** were used to determine the allelic status of the *Gm1*, *Gm2*, *gm3*, *Gm4*, *Gm6, Gm8*, *Gm11*, and *gm12* genes in these genotypes.

PCR-based analysis was carried out in a 20-µl reaction volume containing 1 U of Taq DNA polymerase and 10X PCR buffer. The thermal cycling protocol included an initial denaturation step at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 60 seconds, primer-specific annealing temperature for 60 seconds, extension at 72°C for 2 minutes, and a final extension at 72°C for 7 minutes. The amplified products were resolved using gel electrophoresis on a 3 % agarose gel and visualized under a gel documentation system.

Table 1. Genotypes used and results obtained with gene specific markers for gall midge resistance in rice

S. No	Name of Genotype	Parentage	Reaction to GM*	Marker test for the gene						
				Gm1	*Gm2*	*gm3*	*Gm4*	*Gm8*	*Gm11*	*gm12*
1	TN1 (Susceptible Check)	Dee-Geo-Woogen (DGWG) X Tsai-yuan-chung	Susceptible	-	-	-	-	-	-	-
2	W1263 (Resistant Check RC– *Gm1*)	Eswarakorra X MTU15	Resistant at Ambikapur, Jagdalpur, Ranchi and Jagtial	+						
3	Phalguna (RC-*Gm2*)	IR8 X Siam29	Resistant at Ranchi		+					
4	RP2068-18-3-5 (RC-*gm3*)	Swarnadhan X Velluthachera	Resistant at Ambikapur, Chiplima, Warangal			+				
5	Abhaya (RC-*Gm4*)	CR157-392 X OR57-21	Resistant at Jagdalpur				+			
6	Aganni (RC-*Gm8*)	Land race	Resistant at Ambikapur, Chiplima, Jagtial, Maruteru					+		
7	CR57-MR1523 (RC-*Gm11*)	Ptb21X IR8	Resistant at Ranchi						+	
8	Karma Mahsuri	Mahsuri/ R 296-260 (IET 19910	Resistant at Ambikapur, Jagtial, Ranchi	-	-	-	+	+	+	-
9	PTB20	A selection from V*adakkan chitteni*	Resistant at Ranchi and warangal	-	+	-	+	+	+	-
10	RP 6290-22-53	ISM*2// Abhaya/ Aganni/Swarna	Resistant at Ambikapur, Chiplima, Jagtial, Maruteru, Ranchi	+	-	-	+	+	+	-
11	RP 6749-7-19-16-43	ISM*2// Abhaya/ Aganni/Swarna	Susceptible	+	-	-	+	-	+	-
12	RP 6810-20-41-67-72	ISM*2//RPBio Patho1// Abhaya/Aganni	Susceptible	+	-	+	+	-	-	-

* ICAR-IIRR (2024); + presence of the functional allele; - absence of the allele

Results and Discussion

Linked Marker RM 23956 for *Gm1*

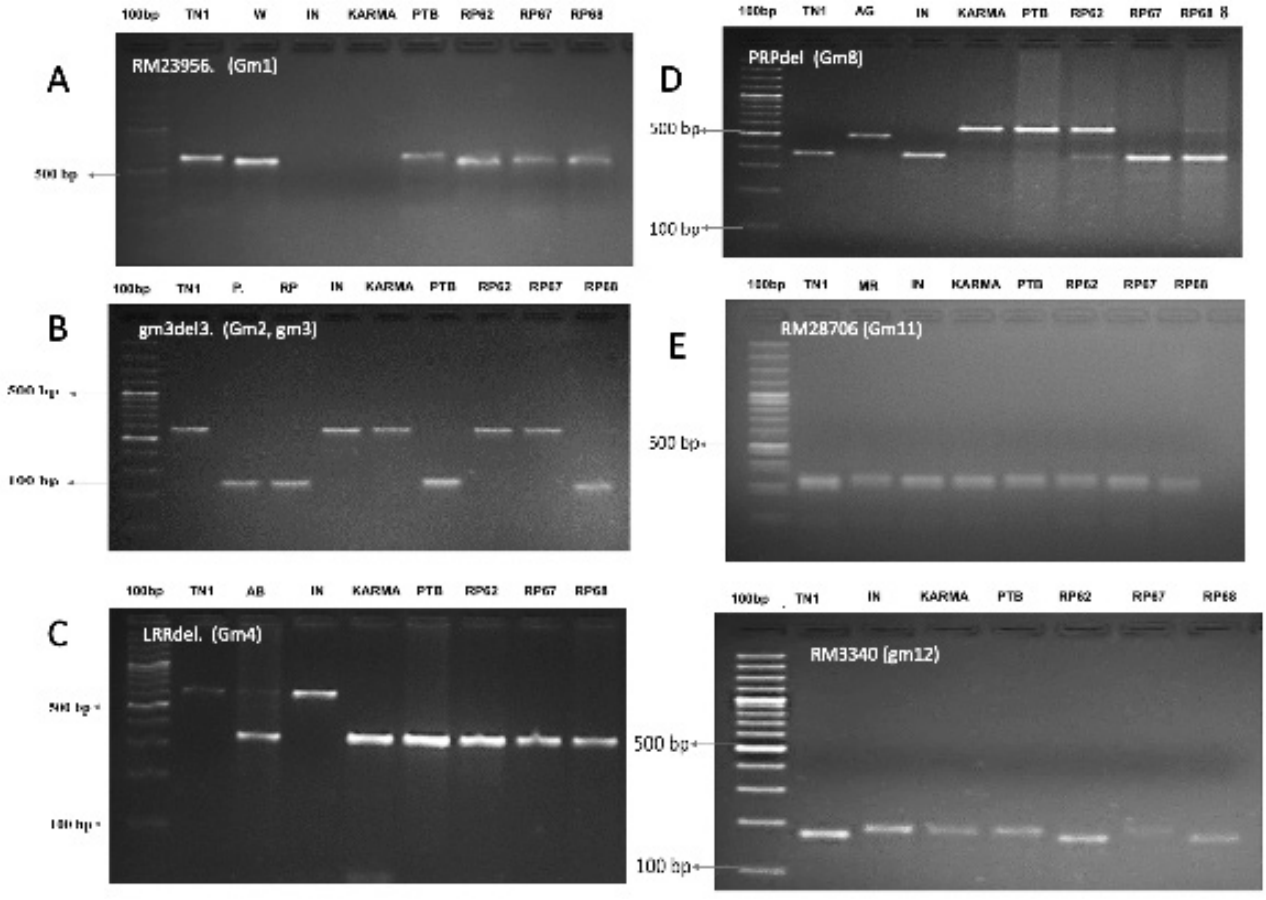
The SSR marker RM 23956 was used to detect the presence of the *Gm1* gene, which imparts resistance to gall midge biotypes 1, 3, 5 and 6 in India (Vijayalakshmi *et al.,* 2006). This marker produced an amplification product of 600 bp in the gall midge-susceptible variety TN1, and of 580 bp in the resistant check variety W1263 (Sundaram, 2007). Among the genotypes analyzed, three genotypes RP 6290-22, RP 6749-7-19, and RP 6810-20 exhibited amplicon size similar to that of W1263, for the flanking marker RM 23956 indicating likely presence of the *Gm1* **(Figure 1A).** In contrast, PTB20 showed amplicon size similar to TN1, suggesting absence of the functional allele. RM 23956 is not a functional marker for this gene but is closely linked to the gene (Sundaram, 2007). Hence a fair degree of recombination between the gene and marker is expected resulting in false positive results. Himabindu *et al*., (2007) suggested to use two flanking markers to detect this gene with greater confidence.

Functional Marker gm3del3 for *Gm2* and *gm3*

The functional marker gm3del3, developed based on sequence polymorphisms in the candidate NB-ARC gene was used to detect the presence of *Gm2, gm3, Gm6* and *Gm7* resistance genes (Sama *et al.,* 2014). This marker differentiates alleles by producing distinct band sizes: a 550 bp in the susceptible check TN1, a 190 bp band in the resistant check Phalguna for *Gm2* gene and a 170 bp band in the resistant check RP 2068-18-3-5 for *gm3* gene. Of the six test genotypes, PTB 20 and RP 6810-20 were identified as carrying the resistant alleles for *Gm2* and *gm3* genes, respectively, Karma Mahsuri, RP 6290-22, and RP 6749-7-19 displayed band patterns similar to the susceptible variety TN1 suggesting presence of non-functional allele of the genes **(Figure 1B)**. These findings reiterate the utility of this functional marker as reported by Suvendhu *et al*., (2014), Sama *et al*., (2014), Divya *et al*., (2015) and Kumar *et al*., (2024). As reported by Sama (2011) *Gm2*, *Gm6* and *Gm7* may all be the same gene reported from different genetic backgrounds.

Functional marker LRRdel for *Gm4*

The LRR del functional marker was developed and employed to identify the presence of the *Gm4* gene, revealing distinct amplification patterns between the resistant (350bp amplicon) and the susceptible (620bp amplicon) sources (Divya *et al.,* 2015). This gene was reported to confer resistance against gall midge biotypes 1, 2, 3, 4 and 4M (Vijayalakshmi *et al.,* 2004). All the five genotypes tested were found to carry the *Gm4* gene allele with specific amplicon size **(Figure 1C).** Based on cloning of the candidate LRR gene (LOC_Os08g09670.1), sequence and functional analysis Divya *et al*., (2015) reported that a deletion of 273 bp region downstream of the gene is the cause of resistance in rice. Karma Mahsuri, a gall midge resistant variety (biotype 1,4 and 5) was released in 2008 for irrigated areas of Chattisgarh and still under cultivation.



Functional marker PRPdel for *Gm8*

The PRPdel functional marker, covering part of the Proline Rich Protein encoding gene, was employed to assess the presence of the *Gm8* gene conferring HR- type resistance against gall midge biotypes 1, 2, 3, 4 and 4M. This marker facilitates the distinction between resistant sources, such as Aganni, and susceptible sources, such as TN1, by generating distinct amplification patterns of 507 bp and 374 bp, respectively, as reported by Divya *et al*., (2018). In the current analysis, none of the genotypes examined exhibited the 507 bp amplicon indicative of the *Gm8* functional allele. However, three genotypes: Karma Mahsuri, Ptb 20 and RP 6290-22 displayed a close allele **(Figure 1D)** that could represent *Gm8* candidate gene LOC_Os08g15080.1 (Divya D, personal communication).

Linked marker RM 28706 for *Gm11*

The molecular marker RM 28706 located within 3.8c M genetic distance of the gene *Gm11*was utilized to detect this gene (Himabindu *et al.,* 2010) that confers resistance against gall midge biotypes 1, 2, 3 and 4. This marker produced an amplification product of over 250bp in the resistant source CR57-MR1523, whereas it generated a product of approximately 200bp in the susceptible source TN1. Among the six genotypes evaluated, five genotypes exhibited banding patterns identical to MR1523 **(Figure 1E).** This similarity suggests that these genotypes are likely to carry the *Gm11* resistance allele with 96 % confidence. On the other hand, RP 6810-20, displayed an amplification pattern consistent with TN1, indicating absence of this allele.However simultaneous use of another linked and flanking marker RM 28574 for detection of the gene improves precision. No candidate gene has been identified for this gene (Himabindu *et al.,* 2010).

Linked marker RM 3340 for *gm12*

The marker RM 3340 was employed to identify the presence of the *Gm12* allele, which is associated with resistance to gall midge in Thailand. This marker produced an amplification product of approximately 170bp in the susceptible source TN1, whereas in resistance source MN62M it was 110bp (Leelagud *et al.,* 2020). Among the six genotypes tested, none amplified 110bp fragment **(Figure 1F).** It is thus obvious that Indian gall midge resistance breeding programs have no access to this resistance gene as of now.

Interestingly, RP 6290-22 and RP 6749-7-19 genotypes were identified as carrying one of the flanking markers for *Gm1,* and *Gm11* resistance genes, and the functional marker for *Gm4,* RP 6810-20 carried *Gm1, gm3* and *Gm4* whereas PTB 20 carried *Gm2, Gm4* and *Gm11* genes **(Table 2)**. We could observe a change in the virulence pattern of the populations and some of the differentials

Table 2. Details of functional/ gene linked markers used for molecular test for presence of the gene

S. No	Name of Gene	Name of Marker	Primer Sequence of Marker (5' --- 3')	Resistant allele size (bp)	Susceptible allele size (bp)	References
1	*Gm1*	RM23956	F- GTCTCTCCCTCTCTCATCTTGTCG R- CCCTATTCATGTGCAATGGAACC	580	600	Sundaram, 2007
2	*Gm2, gm3, Gm6*	gm3del3	F- CTGCCAGAGATGGGCCTTCCA R- CGTACAAATTCCTGTACCACTC	190 (*Gm2*), 170 (*gm3*)	550	Sama *et al.,* 2014 Sama *et al.,*2011
3	*Gm4*	LRRdel	F- GTGGATCGAGAGAAGACAAG R- CTTGAGGACGATATTCAAGC	350	620	Divya*et al.,* 2015
4	*Gm8*	PRPdel	F- TATAAAGAGGACGGTCTAACCTTTA R-GCACAGGGAAGTTGTCAGTTCAAGTA	507	374	Divya *et al.,* 2018
5	*Gm11*	RM28706	F- GGTTCCCGGTCATCATATTTCC R- ACTTTACCCACGCGCTTTGC	250	200	Himabindu *et al.,* 2010
6	*gm12*	RM3340	F- GAGAGAGACACCAAATGATCCATCC R- ACTGATTTGGCCCTTGTTCTTGG	120	150	Leelagud *et al.,* 2020

which were earlier resistant did not show the resistant reaction except for Aganni and W1263 which were conferring resistance across locations as compared to other differentials. It is to be noted that the three RP lines were developed through multiple crosses with many donors as parents. DNA methylation or histone modifications could suppress gene expression, leading to the absence of the phenotype despite the presence of the associated genetic marker. Structural variations such as copy number variations, translocations, or presence/absence polymorphisms (PAVs) may affect the expression of the trait, even if the SSR marker is present (Cedar and Bergman, 2009).

Conclusion

This study successfully utilized reported functional/ linked molecular markers to identify resistance alleles for gall midge resistance genes (*Gm1, Gm2, gm3, Gm4, Gm8, Gm11,* and *gm12*) in various rice genotypes. The findings demonstrated the presence of specific resistance alleles in some genotypes, while others lacked the desired allele of the respective gene. Notably, functional markers such as gm3del3, LRRdel, and PRPdel proved effective in distinguishing resistant and susceptible genotypes, reinforcing their reliability in molecular breeding programs. The genotypes displaying different combinations of the genes have the potential for utilization in developing new rice varieties with durable resistance to gall midge. This advancement could play a significant role in enhancing crop productivity and ensuring food security.

References

Abhilash Kumar V, Balachiranjeevi CH, Bhaskar Naik S, Rekha G, Rambabu R, Harika G, Pranathi K, Hajira SK, Anila M, Kousik M and Kale R. 2017. Marker-assisted pyramiding of bacterial blight and gall midge resistance genes into RPHR1005, the restorer line of the popular rice hybrid DRRH3. *Molecular Breeding,* 37(86):1-14.

Bentur JS, Rawat N, Divya D, Sinha DK, Agarrwal R, Atray I and Nair S (2016). Rice-gall midge interactions: battle for survival. *Journal of Insect Physiology,* 84: 40-49.

Cedar H and Bergman Y. 2009. Linking DNA methylation and histone modification: patterns and paradigms. *Nature Reviews in Genetics*, 10(5): 295-304. DOI: 10.1038/nrg2540.

Divya D, Himabindu K, Nair S and Bentur JS. 2015. Cloning of a gene encoding LRR protein and its validation as candidate gall midge resistance gene, *Gm4*, in rice. *Euphytica,* 203(1): 185-195. DOI: 10.1007/s10681-014-1302-2.

Divya D, Sahu N, Nair S and Bentur JS. 2018. Map-based cloning and validation of a gall midge resistance gene, *Gm8*, encoding a proline-rich protein in the rice variety Aganni. *Molecular Biology Reporter,* 45(6): 2075-2086. DOI: 10.1007/s11033-018-4364-8

Himabindu K, Sundaram RM, Neeraja CN, Mishra B and Bentur JS. 2007. Flanking SSR markers for allelism test for the Asian rice gall midge (*Orseolia oryzae*) resistance genes. *Euphytica*, 157(1): 267-279. DOI: 10.1007/s10681-007-9419-1.

Himabindu K, Suneetha K, Sama VSAK and Bentur JS. 2010. A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. *Euphytica,* 174 (2): 179-187. DOI: 10.1007/s10681-009-0106-2.

ICAR-IIRR 2018-2024. Progress Reports 2017-2023, Vol.2, Crop Protection. (Entomology and Plant Pathology). All India Coordinated Research Project on Rice. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad - 500 030, Telangana State, India.

Katti G. 2021. Overview of entomology research under AICRIP-An experiential learning. *Journal of Rice Research*, 14(2): 69-77, https://doi.org/10.58297/GYTJ6236 .

Kumar A, Hari Y, Suresh J, Srinivas B, Omprakash S, Reddy CN, Pal S, Kumar RS and Edukondalu B. 2024. Molecular confirmation for gall midge (Biotype 3) resistance in phenotypically resistant rice genotypes using functional markers. *Journal of Advances in Biology and Biotechnology,* 27(11): 348-354. DOI: 10.9734/jabb/2024/v27i111620.

Leelagud P, Kongsila S, Vejchasarn P, Darwell K, Phansenee Y, Suthanthangjai A, Uparang C, Kawichai R, Yajai P, Boonsa-Nga K and Chamarerk V. 2020. Genetic diversity of Asian rice gall midge based on *mtCOI* gene sequences and identification of a novel resistance locus *gm12* in rice cultivar MN62M. *Molecular Biology Reports,* 47(6): 4273-4283.

Mathur KC and Krishnaiah K. 2004. Rice gall midge: pest status, distribution, and yield losses. Pp 63-70. In New Approaches to Gall Midge Resistance in Rice. (Edited by Bennett J, JS Bentur, IC Pasalu and Krishnaiah K) International Rice Research Institute, Los Banos, Philippines.

Nair S, Bentur JS and Sama VSAK. 2011. Mapping gall midge resistance genes: towards durable resistance through gene pyramiding. In: Genomics and Crop improvement: Relevance and Reservations. pp. 256-264. Institute of Biotechnology, ANGR Agricultural University, Hyderabad, India.

Padmakumari AP, Kota S and Sundaram RM. 2024. Current Status of Host Plant Resistance to Insects in Rice and Future Perspectives. Pp. 69-122. In Kumar S, Furlong M (eds.) Plant Resistance to Insects in Major Field Crops. Springer Nature, Singapore. ISBN 978-981-99-7519-8ISBN 978-981-99-7520-4 (eBook) https://doi.org/10.1007/978-981-99-7520-4.

Perumalla JR, Vinukonda VP, Singh UM, Alam S, Venkateshwarlu C, Vipparla AK, Dixit S, Yadav S, Abbai R, Badri J, Ram T, Padmakumari AP, Singh VK and Kumar A .2021. Marker-assisted forward and backcross breeding for improvement of elite Indian rice variety Naveen for multiple biotic and abiotic stress tolerance. *PLOS ONE* 16(9): e0256721. https://doi.org/10.1371/journal.pone.0256721.

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard R. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of National Academy of Sciences USA,* 81(24): 8014-8018. DOI: 10.1073/pnas.81.24.8014.

Sama VSAK. 2011. Identification, tagging and mapping new resistance gene(s) against the Asian rice gall midge *Orseolia oryzae* in rice varieties. Ph.D. Thesis submitted to the Osmania University, Hyderabad, pp. 216.

Sama, VSAK, Rawat N, Sundaram RM, Himabindu K, Naik BS, Viraktamath BC and Bentur JS. 2014. A putative candidate for the recessive gall midge resistance gene *gm3* in rice identified and validated. *Theoretical and Applied Genetics,* 127(1): 113-124. DOI: 10.1007/s00122-013-2205-7.

Shilpi D, Singh UM, Singh AK, Alam S, Venkateshwarlu C, Nachimuthu VV, Yadav S, Abbai R, Selvaraj R, Devi MN, Ramayya PJ, Badri J, Ram T, Lakshmi VJ, Lakshmidevi G, Jai VLRK, Padmakumari AP, Laha GS, Prasad MS, Seetalam M, Singh VK and Kumar A. 2020. Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/ tolerance in rice, *Rice*, 13(1): 29 (1-15). https://doi.org/10.1186/s12284-020-00391-7.

Singh B, Mishra S, Bisht DS and Joshi R. 2021. Growing rice with less water: Improving productivity by decreasing water demand. In Rice improvement: physiological, molecular breeding and genetic perspectives. Cham: Springer International Publishing pp. 147-170.

Sundaram RM. 2007. Fine mapping of rice gall midge resistance genes *Gm1* and *Gm2* and validation of the linked markers. Ph.D. thesis submitted to the University of Hyderabad, Hyderabad, pp.181.

Suvendhu DS, Divya D, Rani CD, Reddy TD, Visalakshmi V, Cheralu C, Singh KI and Bentur JS. 2014. Characterization of gall midge resistant rice genotypes using resistance gene specific markers. *Journal of Experimental Biology and Agriculture Sciences, 2*(4): 439-446.

Vijayalakshmi P, Amudhan S, Himabindu K, Cheralu C and Bentur JS. 2006. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. *International Journal of Tropical Insect Science,* 26(03): 207-211, doi: 10.1079/IJT2006114.

Vijay Kumar L, Patil SU, Shivanna B and Kitturmath MS. 2022. Hypersensitive response and induced resistance in rice gene differentials against biotype 1 of Asian rice gall midge, *Orseolia oryzae* at Mandya, Karnataka. *Journal of Rice Research,* 15(1): 70-76, https://doi.org/10.58297/MJYG3539 .

Yang Y, Guo M, Sun S, Zou Y, Yin S, Liu Y, Tang S, Gu M, Yang Z and Yan C. 2019. Natural variation of OsGluA2 is involved in grain protein content regulation in rice. *Nature Communications* 10(1): 1949.

Zhou H, Wang X, Mo Y, Li Y, Yan L, Li Z, Shu W, Cheng L, Huang F and Qiu Y. 2020. Genetic analysis and fine mapping of the gall midge resistance gene *Gm5* in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics,* 133(52): 2021-2033.