Molecular Confirmation for Gall Midge (Biotype 3) Resistance in Phenotypically Resistant Rice Genotypes using Functional Markers

ABSTRACT

This study ~~carried out~~performed molecular screening of rice genotypes, which showed no gall infestation in field conditions at the Regional Agricultural Research Station (RARS), Jagtial, during rabi, ~~the~~ 2021-22. ~~Rabi season~~. Using functional markers such as "gm3del3" for the gm3 gene, "LRR del" for the Gm4 gene, and "PRP" for the Gm8 gene, six genotypes were analyzed. ~~Among them,~~ WGL-1145 contained all three resistance genes (gm3, Gm4, and Gm8). Additionally, three genotypes namely WGL-1147, WGL-1127, and Kakai had the Gm4 and Gm8 genes, while RP-5332-54-11-8-2-13 had only Gm8 and IR72476-B-P-9-3-1-1 possessed only gm3. These findings contribute to ~~the development of~~developing new rice varieties with resistance to gall midge, which can improve crop yields and food security in affected regions. Some promising rice varieties may be used as donors in breeding programs aimed at creating pyramided lines with durable gall midge resistance.

Keywords: Rice, Gall midge resistance, Functional Markers

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple for approximately 40% of the global population, cultivated over 164 million hectares with an annual production of 756.7 million tonnes [1]. Asia dominates production and consumption, with India leading in area (45 million hectares) and ranking second in production (178.3 million tonnes), contributing 23.5% of the global rice supply [1]. Despite significant yields, rice cultivation faces challenges due to pest and disease susceptibility, which hampers productivity. Biotic stress factors account for nearly half of global rice production losses, with insect pests responsible for approximately 25% of these damages [2]. Among the major pests, the stem borer, brown plant hopper, and gall midge are particularly destructive. The gall midge alone causes global damage exceeding US$700 million annually [3].

The rice gall midge comprises two species: the Asian rice gall midge (*Orseolia oryzae*) and the African rice gall midge (*Orseolia oryzivora*). The Asian rice gall midge is particularly problematic, inflicting annual yield losses valued at over US$700 million [3, 4, 5]. In India, this pest contributes to an average annual yield loss of about 4.77 lakh tonnes, equivalent to 0.8% of total production and worth approximately US$80 million [6]. The Asian rice gall midge affects various South and Southeast Asian countries, including Bangladesh, China, India, Indonesia, Myanmar, Sri Lanka, and Vietnam, and is ranked as the third most significant pest in India, following the stem borer and plant hopper [7].

Chemical control methods for gall midge are largely ineffective and environmentally damaging. Thus, cultivating resistant rice varieties offers a more sustainable solution [5]. While several gall midge resistant varieties have been developed and released in India, the widespread use of varieties with single resistance genes has led to the emergence of new virulent biotypes and resistance breakdown [6]. To address this issue, the pyramiding of multiple resistance genes not previously deployed and mechanistically diverse has been proposed [8]. To date, eleven major resistance genes (*Gm1-Gm11*) have been identified with nine of these mapped to rice chromosomes [9]. Among these, four genes- *Gm2*, *gm3*, *Gm4*, and *Gm8* have been functionally validated [10, 11, 12]. Marker-assisted breeding has facilitated the introgression of *gm3*, *Gm4*, and *Gm8* genes into elite rice varieties [13, 4, 5].

2. MATERIALS AND METHODS

Molecular analysis was conducted on six rice genotypes that exhibited 'nil' gall infestation, as detailed in Table 1. DNA was isolated from these six genotypes grown under field conditions at the Regional Agricultural Research Station ~~(RARS)~~, Jagtial, during the ~~*Rabi*~~ *rabi,* ~~season of~~ 2021-

22, following the protocol described by [14]. To determine the allelic status of the *gm3, Gm4,* and *Gm8* genes, functional markers were employed as listed in Table 2. The PCR-based analysis was performed with 1 U of Taq DNA polymerase (Fermentas, Lithuania), 1X PCR buffer (Genei, India), and a 10-µl reaction volume, using a thermal profile of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute and a final extension at 72°C for 7 minutes. Gel electrophoresis was used to resolve amplified products, with *gm3* and *Gm4* genes analyzed on a 1.2% Seakem LE® agarose gel (Lonza, USA), while the amplified products of *Gm8* gene was analyzed on a 3.5% Seakem LE® agarose gel containing 0.5 mg/ml of ethidium bromide in 0.5X TBE buffer, visualized under UV light.

Table 1. Genotypes used for molecular screening and their phenotypic reaction for Gall midge resistance

Comment [AA1]: From where you take data of this table? Acknowledge or cite those authors. If its data from your field trial than provide necessary details in material and methods?

S. No.	Name of the Genotype	Silver Shoot (SS) %		Reaction
		30 DAT	50 DAT	
1.	IR72476-B-P-9-3-1-1	2.14	0.65	R
2.	RP-5332-54-11-8-2-13	1.44	0.52	R
3.	Kakai	0.00	0.00	HR
4.	WGL-1145	0.00	0.00	HR
5.	WGL-1147	0.00	0.00	HR
6.	WGL-1127	0.00	0.00	HR
7.	Agani (Resistant Check)	0.00	0.00	HR
8.	TN 1 (Susceptible Check)	9.25	79.09	HS

HR-Highly resistant, R-Resistant, HS- Highly susceptible, DAT-days after transplanting

Comment [AA2]: References of this resistance classification ?

Table 2. Details of functional markers used for molecular analysis

S. No.	Name of the Gene	Name of the Marker	Sequence of Marker	Resistant allele (bp)	Susceptible allele (bp)	References
1	*gm3*	gm3del3	F-5'CTGCCAGAGATGGGCCTTCCA3' R-5'CGTACAAATTCCTGTACCACTC3'	250	550	[11]
2	*Gm4*	LRR-del	F-5'GTGGATCGAGAGAAGACAAG3' R-5'CTTGAGGACGATATTCAAGC3'	350	600	[15]
3	*Gm8*	PRP	F-5'TCATGTTGTGCAGATCAACC3' R-5'AGCCATATGAAAACCACCAA3'	300	350	[16]

3. RESULTS AND DISCUSSION

Molecular screening was conducted on six rice genotypes that exhibited 'nil' gall infestation in field screenings. For control of gall midge, development of resistant rice varieties using marker assisted selection can be a sustainable and cost-effective approach [17, 18, 19, 20]. Gene pyramiding with two or additional active genes in a single variety may lead to strong gall midge resistance rice varieties. Nowadays, the use of molecular markers for improvement of gene pyramids in desired combination is being monitored in different rice cultivars and presently using DNA markers for selection of resistant plants for gene pyramiding has been accepted as an established tool [21, 17]. In the present investigation, among the molecular markers, we used functional markers, because they are developed from functional gene motifs and therefore, have complete linkage to the desired allele [22]. Due to the complete linkage of an functional markers with the target gene and the absence of recombination between the marker and the gene, the loss of information and the false selection in Marker Assisted Breeding can be prevented [23]. Functional markers can reduce linkage drag, particularly in foreground selection by genotyping a smaller population size [24].

Comment [AA3]: As per comments of table 1

Functional Marker gm3del3 for *gm3* Gene:

The gm3del3 functional marker was utilized to detect the presence of the *gm3* gene, showing different amplification patterns in resistant and susceptible sources. The gm3del3 marker was designed based on sequence polymorphisms in the NB-ARC gene, which is a candidate gene for resistance [11]. This functional marker showed an amplicon size of 550 bp for the susceptible allele and 250 bp for the resistant allele [11]. Among the six genotypes screened, two genotypes namely IR72476-B-P-9-3-1-1 and WGL-1145 were observed to be positives for the *gm3* gene (Figure 1). These findings are consistent with the results reported by [17, 11]. Recent validation through RT-PCR studies [15] has confirmed the efficacy of this marker. Additionally, employed the gm3del3 marker to screen gene pyramided lines containing *Gm1, gm3* and *Gm8* for the presence of the *gm3* gene [4].

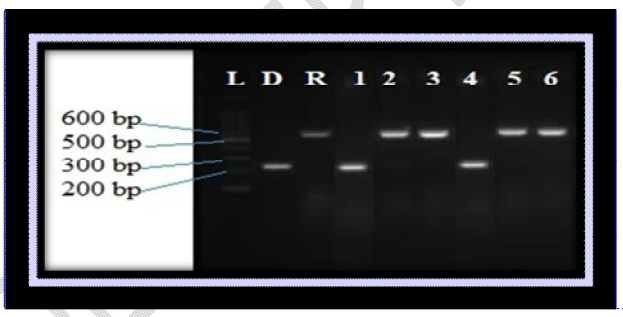

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Figure 1: PCR Amplification pattern of six rice genotypes with *gm3del3* functional marker, screening for presence of *gm3* gene

Figure Legends: L- 100 bp ladder, D- RP2068 (donor parent for g*m3* gene), R-TN1 (susceptible parent for *gm3* gene). The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 1.

Functional Marker LRR del for *Gm4* Gene:

The LRR del functional marker was used to detect the presence of the *Gm4* gene, exhibiting distinct amplification patterns in resistant and susceptible sources. Specifically, this marker amplified a band size of 350 bp in the *Gm4* resistant source Abhaya and 600 bp in the susceptible source TN1 [15]. Out of six genotypes screened, four genotypes namely Kakai, WGL-1145, WGL-1147 and WGL-1127 were observed to be positive for the *Gm4* gene (Figure 2). These results are consistent with the findings of [25, 17]. Earlier, the LRR-del marker was employed by [26] to screen intercrossed F_4 (ICF_4) lines carrying *Gm4, Gm8* and *Xa21* genes and by [27] to screen backcross derived lines for the presence of *Gm4*.

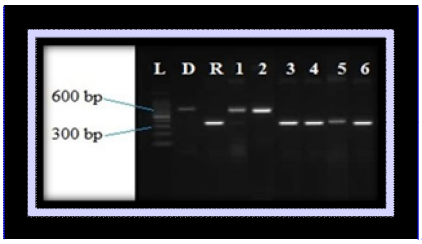

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Figure 2: PCR Amplification pattern of six rice genotypes with *LRR-Del* functional marker, screening for presence of G*m4* gene

Figure Legends: L- 100 bp ladder, D- TN1 (susceptible parent), R-Abhaya (donor parent for *Gm4* gene). The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 1.

PRP-del Functional Marker for *Gm8* Gene:

The PRP marker, which encodes a Proline Rich Protein [16], was used to confirm the presence of the *Gm8* resistance gene in these test entries. PRP marker showed distinct amplification patterns in resistant and susceptible sources. Specifically, this marker amplified a band size of 300 bp in the *Gm8* resistant source Aganni and 350 bp in the susceptible source TN1. Out of six genotypes, five genotypes namely RP-5332-54-11-8-2-13, Kakai, WGL-1145, WGL-1147 and WGL-1127 were observed to be positive for the *Gm8* gene (Figure 3). These findings are consistent with those reported by [16, 17].

Recent validation through RT-PCR studies has confirmed the functionality of this gene [15]. Additionally, the PRP marker has been used by [26] to screen intercrossed F_4 (ICF_4) lines carrying *Gm4, Gm8* and *Xa21* genes for the presence of *Gm8*, by [27] to screen backcross-derived lines with *Gm4, Gm8*, and *Xa21* genes for *Gm8*, and by Venkanna *et al*. (2018) to screen gene pyramided lines with *Gm1*, *gm3*, and *Gm8* for the presence of *Gm8*.

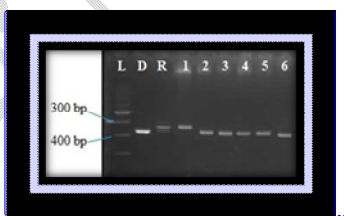

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Figure 3: PCR Amplification pattern of six rice genotypes with *PRP-del functional* marker, screening for presence of *Gm8* gene

Figure Legends: L-100 bp ladder, D- Aganni (donor parent) R- TN1 (susceptible parent for *Gm8* gene). The lane numbers 1-6 written on top of the gel indicates list of genotypes used for molecular screening given in Table 1.

Earlier, our published results [5] indicated that WGL-1145 and WGL-1147 possessed single Gall midge resistance gene *Gm4*. However, further phenotypic and genotypic screening of these two rice genotypes indicated that, WGL-1145 in addition *Gm4* it also possesses other two Gall midge resistance genes i.e. *gm3* and *Gm8*, while WGL-1147 was found to possess other Gall midge resistance gene i.e. *Gm8*.

Based on the current study it can be concluded that, Out of six rice genotypes, WGL-1145 possessed all three resistance genes (i.e. *gm3, Gm4* and *Gm8*) and three genotypes namely WGL-1147, WGL-1127 and Kakai possessing *Gm4* and *Gm8* genes, while RP-5332-54-11-8-2-13 possessed only *Gm8* gene and IR72476-B-P-9-3-1-1 possessed only *gm3* gene. These

results will aid in the creation of new rice varieties resistant to gall midge, enhancing crop yields and food security in impacted areas. Some of the promising rice cultures may be utilized as donors in conventional breeding programs for development of pyramided lines with durable gall midge resistance.

4. CONCLUSION

The study investigates the molecular screening of rice genotypes resistant to gall midge infestations, conducted at the Regional Agricultural Research Station (RARS) during the rabi, 2021-22. Using functional markers for the gm3, Gm4, and Gm8 resistance genes, six phenotypically resistant genotypes were analyzed. Notably, WGL-1145 was found to possess all three resistance genes, while others showed varying combinations of the genes. The findings underscore the potential for developing new rice varieties with enhanced gall midge resistance, contributing to improved crop yields and food security. The research emphasizes the role of marker-assisted breeding in creating durable resistance through gene pyramiding.~~The functional markers revealed that WGL-1145 carries all three resistance genes (gm3, Gm4 and Gm8), while other genotypes showed varying combinations of these genes. These findings are valuable for developing new rice varieties resistant to gall midge, contributing to improved crop yields and food security. Promising genotypes can serve as donors in breeding programs to develop durable, resistant rice lines.~~

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