Original Research Article

Genetic Validation of Promising Warangal Rice Cultures for Gall midge Resistance using Functional Markers

ABSTRACT

The experiment was conducted during *Rabi* 2023-24 at Regional Agricultural Research Station, Warangal. The Asian gall midge *Orseolia oryzae* is one of the major pests of rice which is causing significant economic loss to the crop. Thirty nine rice cultures along resistant checks (Aganni and RMSGM3) and susceptible check (TN-1) were screened for the presence of three gall midge resistance genes namely, *gm3, Gm4* and *Gm8* by using functional markers like gm3del3, LRR-del and PRP, respectively. Out of 39 rice cultures, five rice cultures namely, WGL-1940, WGL-1942, WGL-1963, WGL-1956 and MIL-12 were observed to be triple positives by possessing all the three gall midge resistant genes (i,e.*gm3, Gm4* and *Gm8*), While three rice cultures namely, WGL-1778, WGL-1800 and BM-71 were observed to posses *gm3* and *Gm8* gall midge resistance genes and 14 rice cultures namely, WGL-1781, WGL-2000, WGL-2003. WGL-1941, WGL-1949, WGL-1960, WGL-2038, WGL-1145, INRC-3021, WGL-1124, Aganni, WGL1127, WGL-2039 and WGL-2040 were observed to posses *Gm4* and *Gm8* genes. This research successfully identified gall midge resistance genes in 70% of the rice cultures tested using functional markers. 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 breeding programs for development of pyramided lines with durable gall midge resistance.

Key Words: Rice, gall midge, Molecular Markers,

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a stable food for more than half of the world population, especially in Asia. Approximately 52% of global rice production is annually lost due to the damage caused by biotic stress factors, of which 25% is attributed to the attack by insect pests (Yarasi *et al*, 2008). Among the various insects pests of rice that cause economic losses in south Asia, the rice gall midge ranks third after stem borers and plant hoppers (Bentur et al. 2016). The Asian rice gall midge, *Orseola oryzae* (Wood-Mason) (Diptera: cecidomyiidae) is widely spread in Asia, causing significant yield losses In India. gall midge damage causes an average annual yield loss of about 0.8% of the total production accounting to US $ 80 million (Krishnaiah. 2004). The larva of gall midge feeds on the apical meristem causing the formation of tubular gall called as silver shoot. Galls occurs generally during the tillering stage. Early gall midge infestation results in profuse tillering and stunting but these tillers do not bear panicles resulting in yield losses (Bentur et al. 1987). The best way to manage the pest is the cultivation of resistant varieties. Till date there are seven gall midge biotypes (GMB1, GMB2, GMB3, GMB4, GMB5, GMB6 and GMB4M) were reported (Vijaya Lakshmi et al. 2006) and 12 gall midge resistance genes (designated as *Gm1* to *gm*12) have been identified from different sources (Himabindu et al. 2010; Leelagud et al. 2020), .among them 10 genes, namely *Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm11* and *gm12* were tagged and mapped successfully (Leelagud *et al*, 2020). The marker gm3del3 designed for the candidate gene NB-ARC for *gm3* gene which is located on the chromosome 4 completely co segregated with the trait in the mapping population of 300F 10 RILs (Sama et al. 2014). It was identified that a LRR gene as a candidate gene for *Gm4* based on physical location, structural diversity, co segregation and functional validation also revealed LRR-del as a functional marker which can be used for detecting *Gm4* as this marker produces amplified fragments at 600 bp in TN-1 (susceptible check) and 350 bp in Abhaya (resistance check) (Divya et al. 2014). Further in-silico analysis were made by Yasala et al. (2012) revealing functional gene loci and later attempts narrowed down the search with a candidate gene coding for proline rich protein in the genomic region of *Gm8*, and was further validated and the marker PRP was used for identification of several genotypes with *Gm8* gene (Divya et al. 2015). These three gene based markers have shown a high degree of confidence in detecting the presence of genes in mapping populations (Dutta et al. 2014). With the availability of gene linked markers it is possible to identify the gall midge resistance genes precisely. So the present study was aimed for Genetic Validation of gall midge resistance genes in the rice cultures.

2. MATERIAL AND METHODS

Genetic Validation of phenotypically promising gall midge resistant rice cultures (Table 1) for presence of the 3 gall midge resistant genes (viz, *gm3*, *Gm4* and *Gm8*) by using linked markers or functional markers like Gm3del3, LRR-del and PRP, respectively (Table 1), was carried out at Biotechnology Laboratory, RARS, Warangal during *Rabi,* 2023-24.

DNA was isolated from the promising rice cultures, resistant check (RMSGM3 & Agani) and susceptible check (TN-1) (Table 2) by following the protocol of Zheng et al. (1995). The PCR mixture contained 50 ng template DNA, 5 pmoles of each primer, 0.05 mM dNTPs, 1x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) and 1 unit of Taq DNA polymerase (Fermentas, Lithuania) in a reaction volume of 10µl. Template DNA was initially denatured at 94 ^{o}C for 5 min followed by 35 cycles of PCR amplification with the following parameters: a 30-s denaturation at 94^{o}C, a 30-s annealing at 55^{o}C and 1 min of primer extension at 72^{o}C. A final extension was done at 72^{o}C for 7 min. The amplified products of Gm3del3 (Sama *et al*,2014) LRR-del (Divya *et al ,* 2015) and PRP (Divya *et al,*2013) were electrophoretically resolved on a 1.2 % Seakem LE ® agarose gel (Lonza, USA), containing 0.5 mg/ml of ethidium bromide in 0.5x TBE buffer and visualized under UV.

Table 1: Details of molecular markers used in the study.

S.No.	Name of the Gene	Name of the Marker	Sequence of Marker	Resistant allele (bp)	Susceptible allele (bp)	Reference
1	*gm3*	gm3del3	F-5’CTGCCAGAGATGGGCCTTCCA3’ R-5’CGTACAAATTCCTGTACCACTC3	250	550	Sama *et al.*(2014)
2	*Gm4*	LRR-del	F-5’GTGGATCGAGAGAAGACAAG3’ R-5’CTTGAGGACGATATTCAAGC3’	350	600	Divya *et al.*(2015)
3	*Gm8*	PRP	F-5’TCATGTTGTGCAGATCAACC3’ R-5’AGCCATATGAAAACCACCAA3’	300	350	Divya *et al.*(1013)

3. RESULTS AND DISCUSSION

Genetic Validation of gall midge resistance was carried out for 39 rice cultures along with checks by using functional markers or gene linked markers to know the presence or absence of gall midge resistance genes. Control of rice gall midge, development of resistant rice varieties using marker assisted selection can be sustainable and cost-effective approach (Datta et al. 2014).

Table 2: Genetic Validation of rice cultures for the presences of *gm3*, *Gm4* and *Gm8* genes for gall midge resistance.

S.No.	Name of the rice culture	Genotyping results			Remarks
		gm3	*Gm4*	*Gm8*	
1	WGL-1778	aa	rr	RR	Double positives for *gm3* and *Gm8* genes
2	WGL-1781	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
3	WGL-1782	AA	RR	rr	Positive for *Gm4* gene
4	WGL-1800	aa	rr	RR	Double positives for *gm3* and *Gm8* genes
5	WGL-1989	AA	rr	rr	Negatives for three gall midge resistance genes
6	WGL-1990	AA	rr	rr	Negatives for three gall midge resistance genes
7	WGL-2000	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
8	WGL-2001	AA	rr	rr	Negatives for three gall midge resistance genes
9	WGL-2003	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
10	WGL-2004	AA	RR	rr	Positive for *Gm4* gene
11	WGL-1940	aa	RR	RR	Triple positives for *gm3,Gm4* and *Gm8* genes
12	WGL-1941	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
13	WGL-1942	aa	RR	RR	Triple positives for *gm3,Gm4* and *Gm8* genes
14	WGL-1943	AA	rr	rr	Negatives for three gall midge resistance genes
15	WGL-1944	AA	rr	rr	Negatives for three gall midge resistance genes
16	WGL-1947	aa	rr	rr	Positive for *gm3* gene
17	WGL-1949	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
18	WGL-1956	aa	RR	RR	Triple positives for *gm3,Gm4* and *Gm8* genes
19	WGL-1957	AA	rr	rr	Negative for three gall midge resistance

					genes
20	WGL-1959	AA	rr	rr	Negative for three gall midge resistance genes
21	WGL-1960	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
22	WGL-1962	AA	rr	RR	Positive for *Gm8* gene
23	WGL-1963	aa	RR	RR	Triple positives for *gm3,Gm4* and *Gm8* genes
24	WGL-1964	aa	rr	rr	Positive for *gm3* gene
25	WGL-1969	AA	rr	rr	Negatives for three gall midge resistance genes
26	WGL-2038	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
27	WGL-2041	AA	rr	rr	Negatives for three gall midge resistance genes
28	WGL-1865	AA	rr	rr	Negatives for three gall midge resistance genes
29	WGL-1126	AA	RR	rr	Positive for *Gm4* gene
30	WGL-1145	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
31	INRC-3021	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
32	WGL-1121	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
33	BM-71	aa	rr	RR	Double positives for *gm3* and *Gm8* genes
34	MIL-13	AA	rr	rr	Negatives for three gall midge resistance genes
35	MIL-12	aa	RR	RR	Triple positives for *gm3,Gm4* and *Gm8* genes
36	WGL-1121	AA	rr	rr	Negatives for three gall midge resistance genes
37	WGL-1127	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
38	WGL-2039	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes

39	WGL-2040	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
	Aganni (Resistant Check for *Gm4* & *Gm8* genes)	AA	RR	RR	Double positives for *Gm4* and *Gm8* genes
	RMSGM3 (Resistant Check for *gm3* & *Gm4* genes)	aa	RR	RR	Triple positives for *gm3*,*Gm4* and *Gm8* genes
	TN-1 (Susceptible Check for all three genes)	AA	rr	rr	Negatives for three gall midge resistance genes

Note: RR- presence of Gm4 & Gm8 genes in homozygous dominant condition, rr- absence of Gm4 & Gm8 genes in homozygous recessive condition, while aa - presence of gm3 gene in homozygous recessive condition & AA- absence of gm3 gene in homozygous condition (because gm3 gene is recessive gene and designated with alleles aa or AA),

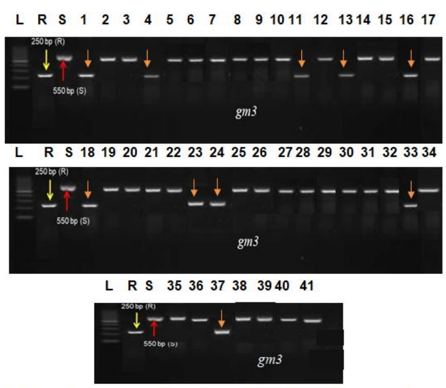
3.1 Genetic Validation for presence of *gm3* gene by using Gm3del3 functional marker

Out of 39 rice cultures (Table 2), 11 rice cultures *viz.* WGL-1778, WGL-1800, WGL-1940, WGL-1942, WGL-1947, WGL-1956, WGL-1963, WGL-1964, BM-71 and MIL-12 were observed to be positives for *gm3* when screened with Gm3del3 functional marker (Table 1) (Figure 1).

The gm3del3 marker was designed based on sequence polymorphism of NB- ARC genes (Sama et al. 2014). It exhibits an allele size of 250 bp for the resistant parent and 550 bp for the susceptible parent (Dutta et al. 2014).

Earlier, Sama et al. (2014) used gm3del3 as a functional marker for introgression of *gm3* gene into the genetic background of the elite bacterial blight resistant cultivar Improved Samba Masuri. Venkanna et al. (2018) employed the gm3del3 marker to screen pyramided lines to determine the presence of the *gm3* gene. Hari et al. (2022) used gm3del3 marker to screen the rice varieties.

Figure 1: Molecular confirmation of the rice cultures for the presence of *gm3* gall midge resistance gene by using gm3del3 functional marker.



The lane numbers (1-42) shown on the top of gel indicates, list of rice cultures used for molecular analysis (Table-1). **L= DNA Ladder (100bp); R=Resistant Check (RMSGM3); S= Susceptible Check (TN1) and arrow mark indicates positive for gm3 gene.**

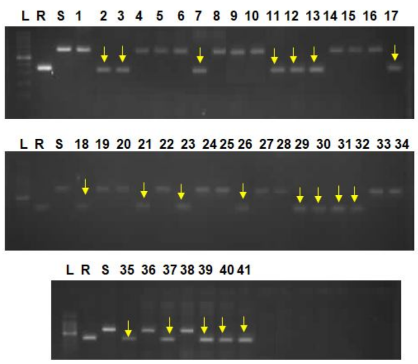
3. 2 Genetic conformation for presence of *Gm4* gene by using LRR-del functional marker

Out of 39 rice cultures (Table 2), 21 rice cultures *viz.* WGL-1781, WGL-1782, WGL-2000, WGL-2004, WGL-1940, WGL-1941, WGL-1942, WGL-1949, WGL-1956, WGL-1960, WGL-1963, WGL-2038, WGL-1126, WGL-1145, INRC-3021, WGL-1124, Aganni, MIL-12, WGL-1127, WGL-2039 and WGL-2040 were observed to be positive for *Gm4* gene when screened with LRR-del functional marker (Table 1) (Figure 2).

The functional marker LRR-del was developed for the identification of *Gm4* gene. The allele size of LRR-del functional marker is 350 bp in resistant parent and 600 bp in susceptible parent (Divya et al. 2015).

Similarly Abhiash Kumar et al. (2017) used the marker to screen inter-crossing F4 cultures that carried the *Gm4* gene.

Figure 2: Molecular confirmation of the rice cultures for the presence of *Gm4* gene by using LRRdel functional marker.



The lane numbers (1-42) shown on the top of gel indicates, list of rice cultures used for molecular analysis (Table 1). **L= DNA Ladder (100bp); R=Resistant Check (RMSGM3); S= Susceptible Check (TN1) and arrow mark indicates positive for Gm4 gene.**

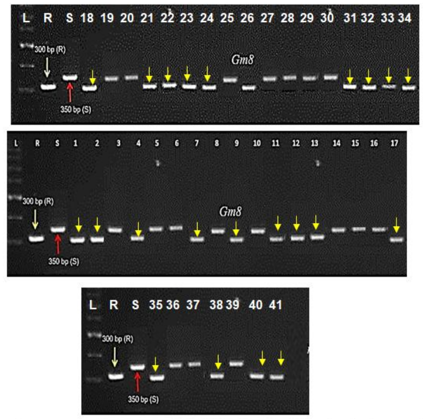
3.3 Genetic conformation for presence of *Gm8* gene by using PRP functional marker

Out of 39 rice cultures (Table 2), 23 rice cultures *viz,* WGL-1778, WGL-1781, WGL-1800, WGL-2000, WGL-2003, WGL-1940, WGL-1941, WGL-1942, WGL-1949, WGL-1956, WGL-1960, WGL-1962, WGL-1963, WGL-2038, WGL-1145, INRC-3021, WGL-1124, BM-71, MIL-12, WGL-1127, WGL-2039, WGL-2040 and Aganni were observed to be positive for *Gm8* gene when screened with PRP functional marker (Table 1) (Figure 3).

The PRP functional marker encoding a Proline Rich Protein was being developed to identify the presence of *Gm8* gene (Divya et al. 2013). The allele size of the PRP marker is 300 bp in the resistant parent and 350 bp in the susceptible parent (Dutta et al. 2014).

Similar to this study earlier Dutta et al. (2014) used this marker to detect the presence of *Gm8* gene with high degree of success. Hari et al. (2022) screened the rice varieties for the presence of *Gm8* using the PRP marker.

Figure 3: Molecular confirmation of the rice cultures for the presence of *Gm8* gene by using PRP functional marker.



The lane numbers (1-42) shown on the top of gel indicates, list of rice cultures used for molecular analysis (Table 1). **L= DNA Ladder (100bp); R=Resistant Check (RMSGM3); S= Susceptible Check (TN1) and arrow mark indicates positive for Gm8 gene.**

4. CONCLUSION

Molecular conformation for gall midge resistance was carried out for 39 rice cultures along with resistant checks (Aganni and RMSGM3) and susceptible check (TN-1) using functional markers to identify the presence or absence of gall midge resistance genes. Out of 39 rice cultures, 5 rice cultures *viz.* WGL-1940, WGL-1942, WGL-1956, WGL-1963 and MIL-12 were observed to be triple positive *i.e.* possessing *gm3, Gm4* and *Gm8 g*all midge resistance genes, 3 rice cultures *viz.* WGL-1778, WGL-1800 and BM-71were observed to be double positive by possessing *gm3* and *Gm8* gall midge resistance genes.14 rice cultures *viz.* WGL-1781, WGL-2000, WGL-2003, WGL-1941, WGL-1949, WGL-1960, WGL-2038, WGL-1145, INRC-3021, WGL-1124, Aganni, WGL-1127, WGL-2039 and WGL-2040 were observed to be double positive by possessing *Gm4* and *Gm8* gall midge resistance genes. 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 breeding programs for development of pyramided lines with durable gall midge resistance.

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COMPETING INTERESTS

"Authors have declared that no competing interests exist."

AUTHORS' CONTRIBUTIONS

THIS WORK WAS CARRIED OUT IN COLLABORATION AMONG ALL AUTHORS. ALL AUTHORS READ AND APPROVED THE FINAL MANUSCRIPT.

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