



SHORT COMMUNICATION

Allele Mining of Major Gall Midge Resistance Genes *gm3*, *Gm4*, *Gm8* and *Gm11* in Selected Sri Lankan Rice Germplasm

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ABSTRACT

Breeding rice varieties carrying resistance to rice gall midge (RGM), *Orseolia oryzae* is a key strategy to reduce yield losses incurred as a result of RGM infestations, globally. Using associated DNA-markers, the study evaluated 55 Sri Lankan rice accessions (23 traditional (TAs) and 32 newly improved rice varieties (NIVs) based on their breeding potential to identify rice accessions carrying resistance alleles at four major RGM resistance genes: *gm3*, *Gm4*, *Gm8*, and *Gm11*. The allele profiling revealed that none of the rice accessions carried resistance alleles for all four genes and five accessions carried only susceptible alleles at the target loci. Eleven accessions carried resistance alleles at gene combinations *Gm4*, *Gm8* and *Gm11* (7), *gm3*, *Gm8* and *Gm11* (3), and *gm3*, *Gm4* and *Gm11* (1). Twenty-four accessions reported combinations of any two resistance alleles from the four target genes, and 15 rice accessions carried only one resistance allele for the three target genes *gm3*, *Gm8* and *Gm11*. The resistance alleles of *Gm11* (56%) and *gm3* (49%) were the most common in the study panel, and the resistance allele of *Gm4* was the least prevalent (33%). Considering all four resistance genes, TAs carried the resistance alleles mostly compared to the NIVs, except in the gene *gm3*. The RGM resistance allele profiling conducted herewith will facilitate taking informative decisions at parental and donor selection for crosses and gene pyramiding, in rice breeding programs. The study must be further expanded with a field evaluation of rice accessions for resistance to RGM and discovering novel resistance genes in the local germplasm to broaden the understanding of RGM resistance in rice.

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The Asian rice gall midge (RGM) *Orseolia oryzae* (Wood-Mason), is one of the major pests of rice reported in several rice-growing countries, including India, China, Thailand, Indonesia and Sri Lanka (Divya et al., 2018). The RGM infected rice plants bends at the leaf tip into a shape of a gall referred to as an "onion tip" or "silver shoot". Since the affected tillers do not attain panicle-bearing, this leads to a considerable yield loss (Venkanna et al., 2018). Even though cultural and biological control methods have been employed, application of chemical pesticides remains the most popular and effective strategy for the control of RGM infestations in rice fields. Unfortunately, long-term and widespread pesticide use contributes to environmental pollution and carries the risk of developing resistant pest biotypes (Yadav, 2010). Therefore, considering both aspects; the efficiency of pest management and environmental impact, breeding and growing resistant varieties have been shown as a most suited strategy to mitigate the field-issues related to RGM (Sharma and Ortiz (2002) and Hooker (1983). To develop resistant varieties, it is crucial to identify and characterize genes conveying resistance to RGM in rice and identify the alleles carried by rice accessions at major RGM resistance genes. These studies facilitate the use of rice accessions as resistance donors in rice breeding programs.

Resistance to RGM is a quantitatively inherited trait and as of now, 12 RGM resistance genes have been identified (Yasala et al., 2012 and Leelagud et al., 2020). Of those, only the genes *gm3* and *gm12* are recessively inherited and the rest were reported as dominant (Divya et al., 2018 and Leelagud et al., 2020). The RGM resistance genes *gm3* (Sama et al., 2014), *Gm4* (Divya et al., 2015) and *Gm8* (Divya et al., 2018) have been cloned and further characterized to deduce their resistance mechanism. Though not cloned and characterized so far, Hamabindu et al., (2010) reported successful utilization of *Gm11* in the development of a RGM resistant variety. The resistance response of RGM genes are specific for biotypes. According to Sarathchandra et al., (2021), biotype I and biotype II has been reported in Sri Lanka. Apart from the

mentioned no other comprehensive study to discover RGM biotypes has been conducted in Sri Lanka. Though much contained due to its island geography, there is a threat of new RGM biotypes getting introduced and also evolving of new biotypes within the country. Such may compromise the resistance in existing rice varieties and futuristic research must be planned towards preparing for such situations. Therefore, when releasing new varieties, a combination of resistance alleles coming from many RGM resistance genes is the best breeding strategy to attain durable resistance to RGM. Hence, as an initial step, this study aims to evaluate the presence of resistance alleles in 55 Sri Lankan rice accessions using SSR markers, specifically focusing on four major RGM resistance genes namely, *gm3*, *Gm4*, *Gm8*, and *Gm11*. By profiling these key RGM resistance genes, we seek to understand the distribution of resistance alleles across traditional accessions (TAs) and newly improved varieties (NIVs) for the utilization in breeding programs aimed at conveying durable resistance to Asian RGM.

Seeds of 55 Sri Lankan rice accessions (23 TAs and 32 NIVs; Supplementary File 1) were grown in seedling trays until the on-set of the three-leaf stage. The DNA was extracted using a modified CTAB method (Doyle and Doyle, 1987) and was normalized to 50 ng/μL.

The rice accessions were genotyped to identify the alleles carried at four major RGM genes *gm3*, *Gm4*, *Gm8* and *Gm11* using published DNA markers (Sama et al., (2014), Divya et al., (2015), Suvendhu et al., (2014), Divya et al., (2018) and Himabindu et al., (2010). Each PCR reaction was carried out in a 15 μL final PCR reaction volume containing, 150 ng/μL template DNA, 1× Gotaq® Green Master Mix (Promega Corporation, Madison, Wisconsin, USA), 0.33 μM forward and reverse primers, volumed up to meet the final concentration using nuclease-free water (Promega Corporation, Madison, Wisconsin, USA). The PCR reactions were conducted in a CT1000 thermal cycler (Bio- Rad, USA) with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60°C for markers

gm3del3, *RM22685* and *RM28574* and 52 °C for 30 s for marker *LRR-del*, with a final elongation at 72 °C for 30 s. The PCR was completed with a final extension step at 72 °C for 5 min. The PCR products were resolved on 3 % (w/v) agarose (Sigma Aldrich, Merck group, Germany) pre-stained with 5% (v/v) ethidium bromide (Sigma Aldrich, Merck group, Germany) and was visualized using a gel documentation system (UVCI-1100, Major Science, USA). The marker profiles for each SSR locus were scored manually based on the amplicon sizes approximated based on 50 bp/ 100 bp DNA ladder (Promega Corporation, Madison, Wisconsin, USA). The resistance alleles and susceptible alleles of each gene were identified based on the previously reported amplicon sizes for the respective alleles (Sama et al., (2014), Divya et al., (2015), Suvendhu et al., (2014), Divya et al., (2018) and Himabindu et al., (2010). The rice accessions that did not yield a PCR product with repeated attempts were identified as 'unknown' in further analysis.

In the current study, allele profiling of 55 Sri Lankan rice accessions was carried using SSR

markers *gm3del3*, *LRR-del*, *RM22685* and *RM28574*, linked to the major RGM resistance genes *gm3*, *Gm4*, *Gm8* and *Gm11*, respectively (Table 2). None of the 55 rice accessions carried the resistance alleles at all four RGM genes considered, emphasizing the absence of alleles conveying resistance to RGM in four out of the 12 known RGM resistance genes in rice. This highlights the importance of gene pyramiding in the studied rice accessions to achieve durable, broad-spectrum resistance against RGM. Eleven accessions carried resistance alleles at combinations of three genes; *Gm4*, *Gm8* and *Gm11* (*Dik wee*, *Mada el*, *Pachchaperumal*, *Kuru wee*, *Kotteyaran*, *Sudu heenati*, and *Bw 302*), *gm3*, *Gm8* and *Gm11* (*Puwakmalatasamba*, *Bg 369* and *Bg 360*) and *gm3*, *Gm4* and *Gm11* (*Batapola el*). Twenty-four accessions reported combinations of any two resistance alleles from the genes *gm3*, *Gm4*, *Gm8* and *Gm11* and 15 rice accessions reported only one resistance allele with respect to all target genes except *Gm4*. Interestingly, rice accessions *Murungakayan*, *Herathbanda*, *Bg 251*, *Ld 408*, and *Bw 272-6b* reported susceptible alleles at all four target RGM resistance loci.

Table 1: Details of gene specific markers used for the amplification of resistance and susceptible alleles of major gall midge resistance genes *gm3*, *Gm4*, *Gm8* and *Gm11* in rice

Gene	Chromosome number	Marker	Forward and Reverse sequence (5'-3')	Expected band size (bp)		Source
				Resistant allele	Susceptible allele	
<i>gm3</i>	4	<i>gm3del3</i>	F:CTGCCAGAGATGG GCCTTCCA R:CGTACAAATTCCT GTACCACTC	250	550	Sama et al., (2014)
<i>Gm4</i>	8	<i>LRR-del</i>	F:GTGGATCGAGAGA AGACAAG R:CTTGAGGAACGAT ATTCAAGC	350	600	Divya et al., (2014)
<i>Gm8</i>	8	<i>RM22685</i>	F:ATGGGCTTCCAGG CTCAATCTCG R:CCCACTCTCACGT CTCCTCTCTTCC	230	210	Divya et al., (2018)
<i>Gm11</i>	12	<i>RM28574</i>	F:TAGTTTGGTGAAG TGGCATTGG R:ATAGTAGGGCAAG GATTCAGAAGAGG	500	450	Himabindu et al., (2010)

The classification of Bw 272-6b and Ld 408 as carrying susceptible alleles in the current study aligns well with its rice varietal descriptor (Rice Research Development Institute, 2024), where Bw 272-6b has been consistently classified as susceptible (S) and Ld 408 classified as moderately susceptible (MS). According to the descriptors, the varieties Bg 251 is classified as moderately resistant (MR) or resistant (R), however, Bg 251 carries susceptible alleles for all four studied RGM resistance genes. The observed field resistance could be conveyed by other known RGM genes and/or novel resistance genes. Interestingly in the descriptors, accessions Bg 369 and Bg 360, were classified as MR, and these carried resistance alleles at

three RGM resistance genes (*gm3*, *Gm8*, and *Gm11*). As popular varieties, their resistance to RGM can be further improved by profiling the other RGM genes and introgression of any lacking resistance alleles to cover a broad range of RGM resistance. However, Bw 302, classified as moderately susceptible (MS), also carried resistance alleles at three RGM resistance genes (*Gm4*, *Gm8*, and *Gm11*), indicating that while the allele combination is promising, not all genes equally contributes to confer resistance in field conditions. Nevertheless, as mentioned before, the differences observed could be a cumulative impact of the allele profile of other RGM resistance genes and novel genes that are still to be identified.

Table 2: Allele profile of 55 Sri Lankan rice accessions for rice gall midge resistance genes *gm3*, *Gm4*, *Gm8* and *Gm11*

Rice Accessions	<i>gm3</i>	<i>Gm4</i>	<i>Gm8</i>	<i>Gm11</i>
	<i>gm3del3</i>	<i>LRR-del</i>	<i>RM22685</i>	<i>RM28574</i>
<i>Dik wee</i> , <i>Mada el</i> , <i>Pachchaperumal</i> , <i>Kuru wee</i> , <i>Kotteyaran</i> , <i>Sudu heenati</i> and Bw 302	-	✓	✓	✓
<i>Batapola el</i>	✓	✓	-	✓
<i>Puwakmalatasamba</i> , Bg 369 and Bg 360	✓	-	✓	✓
<i>Heen karayal</i> , <i>Goda heenati</i> and <i>Wannidahanala</i>	-	✓	-	✓
<i>Galpa wee</i> and At 354	-	-	✓	✓
<i>Maa wee</i> , <i>Sudugoda wee</i> and <i>Pokkali</i>	-	✓	✓	-
<i>Kalubaala wee</i> , <i>Podihatatha</i> , Ld 365 and Bw 372	✓	-	✓	-
<i>Kalu heenati</i> , <i>Devareddiri</i> and Bw 400	-	✓	-	✓
At 303, Ld 253, Bg 301, At 362, Ld 368, Bg 352, At 373 and Ld 371	✓	-	-	✓
<i>Kahata wee</i>	✓	✓	-	-
<i>Dahanala</i> , At 311, Bg 94-1 and Bg 357	-	-	-	✓
Bg 359	-	-	✓	-
Bw 363, Bw 367, Bg 310, Bg 300, Bg 366, Bw 361, Bg 305, Bg 358, At 307 and At 308	✓	-	-	-
<i>Murungakayan</i> , <i>Herathbanda</i> , Bg 251, Ld 408 and Bw 272-6b	-	-	-	-

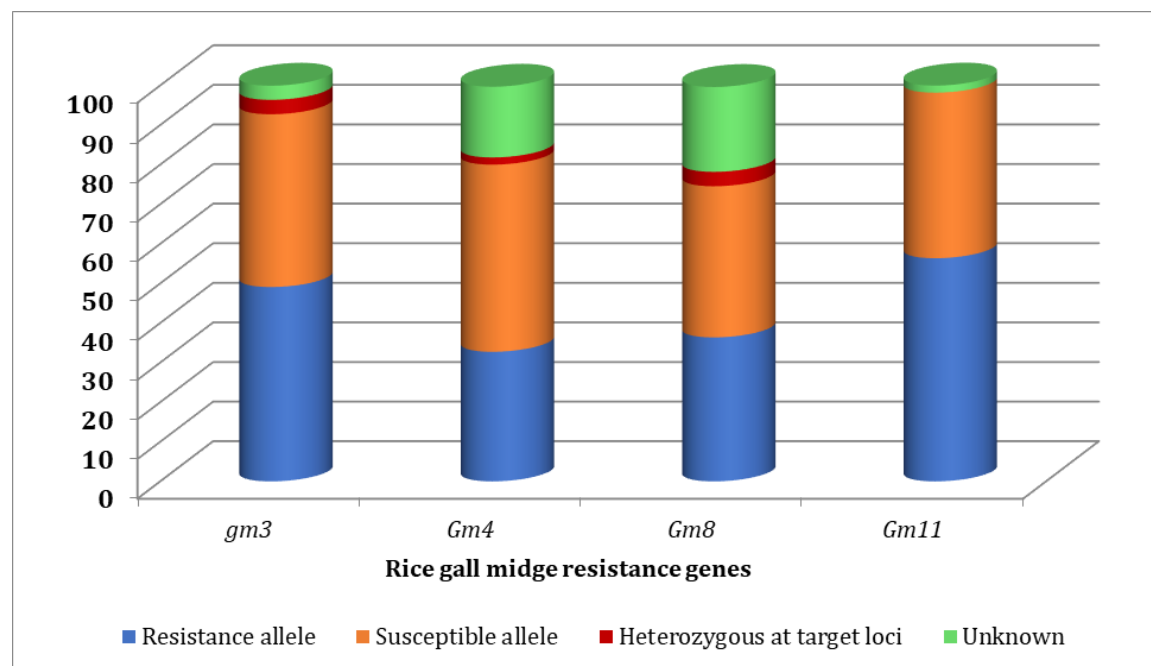


Figure 1: Occurrence frequency of resistance alleles of rice gall midge resistance genes *gm3*, *Gm4*, *Gm8*, and *Gm11* in 55 Sri Lankan rice accessions

Further, according to Figure 1, resistance alleles of *Gm11* (56%) and *gm3* (49%) were the most commonly found in the study panel, and the resistance allele of *Gm4* was the least prevalent (33%). Therefore, all resistance alleles of RGM genes *gm3*, *Gm4*, *Gm8* and *Gm11* are not present in a considerably higher occurrence frequency (<56%) within the study panel. Further, in the current study panel, it was observed that out of the confirmed resistance alleles 19%, 89%, 65% and 48% were observed in the TAs for the RGM resistance genes *gm3*, *Gm4*, *Gm8* and *Gm11*, respectively. Therefore, considering all four RGM resistance genes TAs carry higher proportion of resistance alleles compared to NIVs, except in the gene *gm3*, directing towards a potential for further improvement in the Sri Lankan rice germplasm.

The rice accessions carrying resistance alleles at major RGM resistance genes are of importance in breeding programs as donors of resistance alleles against RGM for gene pyramiding and for the use in backcrosses or as parents in the development of new rice varieties enabling them to withstand RGM infestations under diverse environmental conditions and a broad spectrum of biotypes. The identification of these promising

accessions aligns with the requirements of modern day rice breeding programs and highlights the genetic diversity and potential of Sri Lankan rice accessions as a source for exploring RGM disease resistance. Therefore, this study highlights the importance of integrating allele profiling with phenotypic evaluations for comprehensive resistance characterization of the local rice germplasm for effective utilization in breeding programs. Future research should be expanded to profile alleles of all RGM resistance genes and validate the responses of the identified allele combinations in terms of RGM resistance under field conditions, and discover additional resistance genes to broaden the genetic basis of resistance.

Thirty-five Sri Lankan rice accessions assessed in the study carried resistance alleles for two or three combinations of the four genes *gm3*, *Gm4*, *Gm8*, and *Gm11*, highlighting their genetic potential for RGM resistance. Out of the four genes studied, TAs carried more resistance alleles than NIVs for the RGM resistance genes *Gm4*, *Gm8*, and *Gm11*. The rice accessions carrying multiple resistance alleles for the target genes can be identified as potential donors for gene pyramiding and as parents for crosses in

breeding programs to develop rice cultivars with durable resistance to RGM.

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Supplementary File 1 - Pedigree information, accession numbers , category and source of rice panel

Rice Accession	Pedigree/ PGRC Accession Number	Category	Source
Bg 300	Bg367-7// IR841/ Bg276-5	Newly Improved Variety	Bathalagoda RRDI
Bg 358	Bg12-1/ Bg1492	Newly Improved Variety	Bathalagoda RRDI
Bg 359	88-5089/ Bg379-2	Newly Improved Variety	Bathalagoda RRDI
Bg 360	84-3346/ IR36/ Senerang	Newly Improved Variety	Bathalagoda RRDI
Bg 366	Bg300/ 94-2236//Bg300/Bg304	Newly Improved Variety	Bathalagoda RRDI
Bg 369	Bg 94-1/Nonabokra	Newly Improved Variety	Bathalagoda RRDI
At 303	At66/ Bg276-5	Newly Improved Variety	Bombuwela RRDI
At 307	Bg 2225-3/Bg96-3298	Newly Improved Variety	Bombuwela RRDI
At 308	Bg2225-1/Bg2426-2	Newly Improved Variety	Bombuwela RRDI
At 311	At306/At 03-105	Newly Improved Variety	Bombuwela RRDI
At 362	At 85-2/Bg 380	Newly Improved Variety	Bombuwela RRDI
At 373	IR70422-66-5-2/Bg98-2571	Newly Improved Variety	Bombuwela RRDI
At354	Bg 94-1/Pokkali	Newly Improved Variety	Bombuwela RRDI
Bg 251	ZX788(CNI28)- Introduction from China	Newly Improved Variety	Bombuwela RRDI
Bg 301	1280/ H-4	Newly Improved Variety	Bombuwela RRDI
Bw 302	Bw259-3/Bw 242-5-5	Newly Improved Variety	Bombuwela RRDI
Bg 305	Bg1203/ Bg1492	Newly Improved Variety	Bombuwela RRDI
Bg 310	Bg 300/Pokkali	Newly Improved Variety	Bombuwela RRDI
Bg 352	Bg380/ Bg367-4	Newly Improved Variety	Bombuwela RRDI
Bg 357	Bg797/ Bg300/ 85-1580/ Senerang M-17	Newly Improved Variety	Bombuwela RRDI
Bg 94-1	IR262/ Ld66	Newly Improved Variety	Bombuwela RRDI
Bw 272-6b	Bw259-3/ Bw242-5-5	Newly Improved Variety	Bombuwela RRDI
Bw 363	IR 36/Bw 267-3-11-M	Newly Improved Variety	Bombuwela RRDI
Bw 367	Bw 361/Bw 372	Newly Improved Variety	Bombuwela RRDI
Bw 400	Bw259-3/ Bw242-5-5	Newly Improved Variety	Bombuwela RRDI
Bw 361	IR 36/Bw 267-3-11-M	Newly Improved Variety	Bombuwela RRDI
Bw 372	Bw359/ Bw267-3	Newly Improved Variety	Bombuwela RRDI
Ld 253	Selection from At 4	Newly Improved Variety	Bombuwela RRDI
Ld 365	Selection of Ld 355	Newly Improved Variety	Bombuwela RRDI
Ld 371	Ld 99-11-48/Bg 96-1520	Newly Improved Variety	Bombuwela RRDI
Ld 408	At 01/Ld 98-152	Newly Improved Variety	Bombuwela RRDI
Ld 368	Ld 99-14-11-41/Ld 365	Newly Improved Variety	PGRC
<i>Dahanala</i>	2049	Traditional Accession	PGRC
<i>Godaheenati</i>	4049	Traditional Accession	PGRC
<i>Kottaaran</i>	4630	Traditional Accession	PGRC
<i>Suduheenati</i>	2088	Traditional Accession	PGRC
<i>Wannidahanala</i>	2053	Traditional Accession	PGRC

<i>Herathbanda</i>	2063	Traditional Accession	PGRC
<i>Kaluheenati</i>	2087	Traditional Accession	PGRC
<i>Batapola el</i>	2105	Traditional Accession	PGRC
<i>Dikwee</i>	2109	Traditional Accession	PGRC
<i>Pachchaperumal</i>	3136	Traditional Accession	PGRC
<i>Kalubaala wee</i>	3158	Traditional Accession	PGRC
<i>Podihatatha</i>	3174	Traditional Accession	PGRC
<i>Kuru wee</i>	3254	Traditional Accession	PGRC
<i>Devareddiri</i>	3256	Traditional Accession	PGRC
<i>Murungakayan</i>	3257	Traditional Accession	PGRC
<i>Pokkali</i>	3251	Traditional Accession	PGRC
<i>Galpa wee</i>	3341	Traditional Accession	PGRC
<i>Sudugoda wee</i>	3477	Traditional Accession	PGRC
<i>Puwakmalata samba</i>	3486	Traditional Accession	PGRC
<i>Maa wee</i>	3618	Traditional Accession	PGRC
<i>Kahata wee</i>	3712	Traditional Accession	PGRC
<i>Heenkarayal</i>	4404	Traditional Accession	PGRC
<i>Mada el</i>	3269	Traditional Accession	PGRC

PGRC- Plant Genetic Resource Center

RRDI- Rice Research Development Institute