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Gall midge Resistance Gene Identification in Promising Rice Genotypes using Gene Specific Markers

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ABSTRACT: Rice gall midge, *Orseolia oryzae* (Wood-Mason) attacks shoots of rice crop which results in forming galls impairing growth and yield. The development of resistant varieties and their cultivation is an important strategy to control rice gall midge. Of the 105 lines tested including the susceptible check against ARGM under field conditions during late *kharif*, 2015-16 and 2016-17, the entries,RGL-7002, RGL-1, RGL-7003, RGL-7009 andRGL-7010 recorded the damage of less than 10%. These lines were genotyped for the presence of resistant genes using SSR markers. The entry RGL 7003 possessed the alleles for *Gm1* and *Gm8*while RGL 7002 had *Gm1*and RGL 1 had alleles for *Gm 8*gene.All three of these entries have the potential to be used as donors in breeding trials.

Keywords: Asian rice gall midge, Gene specific markers

INTRODUCTION

India has the world's largest area under rice cultivation and is one of the largest producers of white rice, accounting for 20 per cent of world's rice production. The Asian Rice gall midge, *Orseolia oryzae* is a major insect pest of rice crop. The maggots feed on the apical meristem of the growing shoot resulting in the transformation of leaf sheath into a tubular shoot called 'silver shoot'. Such shoots does not produce inflorescence. The virulence of this pest is such that under favourable conditions the damage due to gallmidge ranged from 23.01 per cent and 96.67 per cent in the susceptible check TN 1(Harathi *et al.,* 2019). Lima *et al.,* (2007) reported the annual yield loss of about 25% over an area of 2, 00,000 ha due to incidence of rice gall midge.
vary from 28% to 35% and has been reported to be 25% over an area of 200,000 ha.
In India, annual yield losses due to gall midge insects have been calculated which vary from 28% to 35% and has been reported to be 25% over an area of 200,000 ha.
Exploiting host plant resistance to RGM is a cost-effective and eco-friendly method for its management (Khush, 1977). Since 1975, around 70 varieties which were resistant to gall midge have been released for commercial cultivation in the pest endemic areas in India (Bentur *et al.,* 2003).
Growing of resistant varieties expressing a single resistance gene over years resulted in the emergence of virulent populations of the pest, known as biotypes, which are capable of overcoming resistance and cause massive losses. The emergence of new and virulent biotypes of the rice gall midge is the reason of concern, as most of the popular gall midge resistant varieties have lost their resistance. In such a setting, new breeding tactics are required to tackle the challenge of durable gall midge resistance. In this study, specific gene-based markers were used for determining the existence of the respective gene in resistant lines.

MATERIALS AND METHODS

Field evaluation of 105 entries were carried out during 2015-16 and 2016-17 at ARS, Ragolu and ARS, Nellore against rice gall midge. Of which five entries recorded plant damage of less than 10. These entries were selected for genotyping.
Ten days aged seedlings of each resistant test line were grown in Petri dish under laboratory condition for extraction of DNA during 2018. CTAB (Cetyl Tri methyl Ammonium Bromide) method was used to extract rice genomic DNA from rice seedlings with slight modifications as followed by Dutta *et al.,* (2014).

Analysis using SSR markers

PCR amplification of the DNA was done using flanking SSR markers and scoring was done based on the presence or absence of the expected gene specific alleles which confer resistance to gall midge *viz*., *Gm1, gm3, Gm4, Gm8,* and *Gm11*.
The PCR products were subjected to agarose gelelectrophoresis. Higher concentration of agarose in the gel facilitates fine separation of small fragments of DNA. Hence, Agarose gel (3%) was used for this study.

Gel documentation

DNA banding pattern was viewed in a UV gel documentation system (Bio-Rad, India) and size of the amplified product was computed using Bio-Rad software. The size of the amplified fragments was determined using 100 bp DNA ladder (reference standard).

RESULTS AND DISCUSSION

Among the 105 field evaluated test lines, five lines recorded(RGL-7002, RGL-1, RGL-7003, RGL-7009 and RGL-7010)per cent plant damage of<10, against rice gall midge were selected for genotyping to record the presence of any reported resistance genes *Gm1*, *gm3*, *Gm4*, *Gm8* and *Gm11* (Table 1). Seven gene specific markers attributing to 5 resistance genes were selected to test the presence of the resistant genes.

Table 1: List of entries selected for Genotyping.

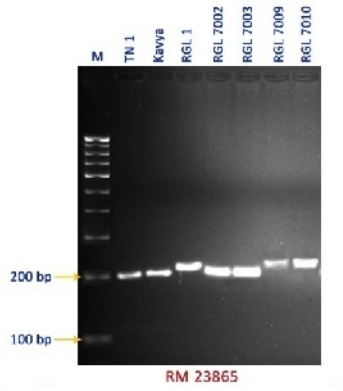
S. No.	Entry	Per cent Plant damage due to rice gall midge			
		2015-16		2016-17	
		30 DAT	50 DAT	30 DAT	50 DAT
1	RGL-1	2.50	6.50	0.00	5.8
2	RGL-7002	5.00	7.50	0.00	7.37
3	RGL-7003	7.5	8.75	0.00	7.19
4	RGL-7009	0.00	5.00	0.00	5.83
5	RGL-7010	0.00	2.50	4.50	6.35

Gm1 gene specific markers

The SSR marker, RM23956 specified for *Gm1* gene, amplified at expected band size of ~610 bp in TN 1 (susceptible check) and at ~990 bp in the resistant donor (W 1263) and none of the entries amplified at ~990 bp or at 610 bp. The band size among the entries ranged from ~580 to 590 ~bp with no amplification in RGL 7003.

Another *Gm1* gene specific marker RM23914 was also amplified for its presence in the test entries and compared with resistant and susceptible check, the banding pattern was not clear among all the rice genotypes compared. In W1263 and TN 1, this marker amplified at ~165 bp and ~100 bp products respectively. The entries, RGL 1, RGL 7003, RGL 7009 and RGL 7010 amplified similar to W 1263, except for the line RGL 7002, which amplified at 165 bp.

Another gene specific marker RM23865 was amplified at a band size of ~200 bp in Kavya, similar banding pattern was observed in RGL 7002 and RGL 7003 (Plate 1).

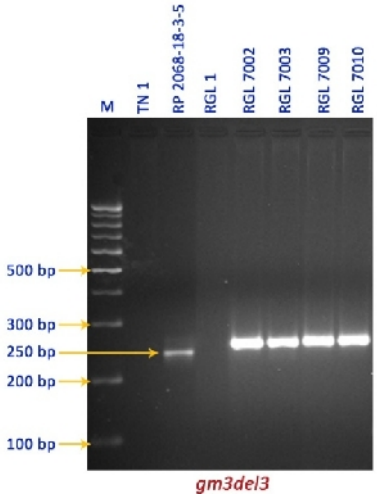


M: Marker - 100bp

Plate 1: Amplification pattern for the presence of RM 23865 marker of the *Gm1* in specified genotypes.

gm3del3 gene specific marker

This marker amplified at a band size ~250 bp in RP2068-18-3-5 (resistant source) and in all the selected entries the amplification was just above the specific site of ~250 bp revealing the absence of specific resistant site in the entries. The amplification of RP 2068-18-3-5 is in agreement with Anusha (2017) and Dutta *et al.* (2014) (Plate 2).

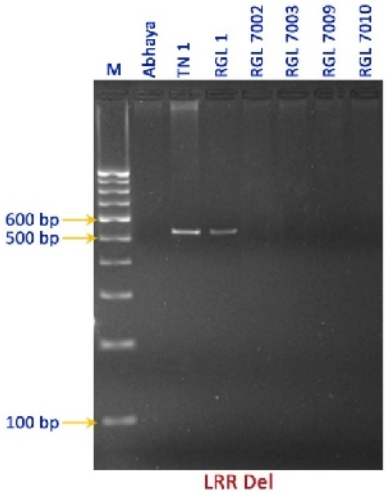


M: Marker - 100bp

Plate 2: Amplification pattern for the presence of *gm3del3*marker of the *gm3* in specified genotypes.

Gm4gene specific marker

LRR Del: This marker amplified the susceptible specific allele (TN 1) at a band size of ~550 bp in RGL 1 and this site is absent in all the other selected entries. The amplification of the marker with respect to susceptible specific allele is in agreement with Divya *et al.* (2014) (Plate 3).

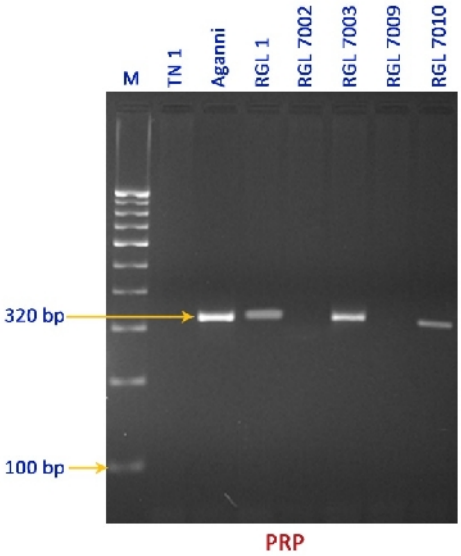


M: Marker - 100bp

Plate 3: Amplification pattern for the presence of LRR Del marker of the *Gm4* in specified genotypes.

Gm8gene specific marker

PRP: This marker amplified at a band size of ~320 bp in Aganni (resistant check) and in the entries RGL 1 and RGL 7003. (Plate 4).



M: Marker - 100bp

Plate 4: Amplification pattern for the presence of the PRP marker of the *Gm8* gene in specified genotypes.

Gm11gene specific marker

In the present study, RM 235 and RM 28574 were used to identify the *Gm11* gene in rice genotypes. Amplification of these genes was not observed in the entries tested, none of the selected entries were found to possess *Gm11*. In contrary, Himabindu *et al.*,(2010) tagged and mapped the new gall midge resistance gene, *Gm11*in CR57-MR1523 using SSR markers, RM 235, RM28574, RM28706, RM17, RM28564 and RM28784 onchromosome 12.

In this study, amplification was observed in resistant check (Kavya), susceptible check (TN 1) and in all selected test entries with RM 23865. Amplification of RM 23865, similar to Kavya was found in RGL 7002 and RGL 7003. When the test entries were amplified with *gm3del3*, amplification was observed in all the entries except for RGL-1, but at a different site from target amplification. Of the 5 selected entries, bands similar to resistant entries were found only in RGL1 and RGL 7003(presence of Gm8 gene specific marker at ~320 bp as in Aganni) and RGL 7003and RGL 7002 for the presence of Gm1 gene similar to Kavya at ~200 bp. In case of RGL 1, Gm8 was amplified similar to resistant check, while in other selected entries new genes might be present which confer resistance against gall midge and can be explored for developing gall midge resistant genotypes. Sama *et al.*, (2012) reported that the markers RM22685 and RM22709 closely flank the *Gm8*gene and this gene was mapped within a 400 kbp region to chromosome 8. These closely linked markers were used to identify nine other gall midge-resistant genotypes.

The use of resistant genotypes as a management strategy for crop protection is not only cost-effective but also environmentally safe. The combination of multiple resistance genes in a single genotype could result in an accumulation of these residual effects. In reality, the success of this strategy is due to more than one mechanism. Therefore, resistance will remain effective far longer than when genes are deployed singly.

CONCLUSION

Genotyping with *Gm1* linked gene specific marker RM23865 revealed the presence of *Gm1* gene in RGL 7002 and RGL 7003. Likewise, with PRP specific marker, the presence of *Gm* 8 was found in RGL 1 and RGL 7003 similar to Aganni. In the current study, RGL-7003 possessed the genes, *Gm1* and *Gm8* gene and it is identified as the new source of resistance against gall midge and the other two lines can also be utilized in breeding experiments.

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