**29. Mapping of a rice gall midge resistance gene, *gm3,* in RP 2068-18-3-5 and *in silico* identification of candidate gene(s)**

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The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) is a major pest of rice causing severe yield losses in several Asian countries like Bangladesh, China, India, Indonesia, Myanmar, Sri Lanka and Vietnam. Host plant resistance is the most suitable approach to manage this pest. However, host plant resistance is often overcome by emergence of new virulent biotypes of the pest.

Genetic studies have identified 11 major genes conferring resistance to gall midge (Kumar et al., 2005 Himabindu et al., 2009). Of these, eight genes (*Gm1, Gm2, Gm4, Gm5, Gm6, Gm7, Gm8 and Gm11*) have been tagged and mapped (Jain et al., 2004 Himabindu et al., 2009). So far seven distinct gall midge biotypes have been characterized (Vijaya Lakshmi et al., 2006). The breeding line RP 2068-18-3-5 derived from the cross Swarnadhan x Velluthacheera has been reported to carry the single recessive gene, *gm3,* (Sahu et al., 1990) conferring resistance against all the gall midge biotypes except GMB5 and GMB6 (Bentur et al., 2009). We report, for the first time, fine mapping of *gm3* gene on chromosome 4 using SSR markers.

Two sets of mapping populations were used in the study. One set consisted of $F_2$ plants (n=112) derived from the cross TN1 (gall midge susceptible) X RP2068-18-3-5 and the second set consisted of 300 recombinant inbred lines (RILs) of the same cross in $F_8$- $F_9$ generations. Both $F_2$ plants and $F_9$ RILs were phenotyped for gall midge resistance in the greenhouse against GMB4. The $F_2$ plants segregated in a phenotypic ratio of 1 :3 (26 R : 86 S) ($\chi^2 = 0.19$, P = 0.67), confirming the report of Sahu et al. (1990).

A total of 490 SSR markers were selected based on their uniform distribution across the rice genome and their hyper-variability in order to detect polymorphism between the parental lines. Of these, 98 markers observed to be polymorphic were used for further analysis of segregating lines. Marker-trait co-segregation analysis in the 112 $F_2$ mapping population revealed two SSR markers RM17473 and RM17480 located on chromosome 4L to be linked to *gm3* at a genetic distance of ~ 3.2 cM and ~ 2.0 cM, respectively. Fine mapping analysis was carried out with 300 progeny tested $F_8$ RIL populations using the set of eight parental polymorphic SSR markers located in the genomic region limited by RM17473 and RM17480. Analysis of data revealed that a specially designed SSR marker, RMgm3SSR4, was very close to the gene with no recombinants. The F and R primer sequences of the marker are given below:

Forward: AGACACGAGGGAATTATGC
Reverse: CTCTATATTTGCCGCATCC

In order to identify putative candidate gene(s) associated with *gm3*, a 200 kb genomic region encompassing RMgm3SSR4 locus was downloaded from the International Rice Genome Sequence database and was analyzed *in silico* for putatively expressed genes using the software tool FGenesH (http://www.softberry.com). A total of 22 putatively expressed genes were identified in the downloaded sequence and among these, a gene encoding a NBS-LRR domain containing protein appeared to be the most probable candidate for *gm3* based on its physical proximity to RMgm3SSR4. Targeting this gene, which is ~ 7 kb in size and consisting of nine exons, five PCR primer pairs were designed for PCR amplification of the resistant (i.e. RP2068) and susceptible (i.e. TN1) parents. One of the primer pairs, targeting the seventh and eighth exons of the gene showed polymorphism among the parents and displayed complete co-segregation with trait-phenotype in RIL population (n=300) (Fig. 1), indicating that this gene could be the putative candidate for *gm3* and the primer pair gm3NBS-LRR5 could serve as a functional marker for the gene for use in Marker Assisted Selection (MAS).

It is interesting to note that the genetic position of *gm3* is also close to the other two major gall midge resistance genes, *Gm6* and *Gm2,* reported earlier (Katiyar et al., 2001 Sundaram, 2007). With the identification of markers linked to *gm3* in this study, major gall midge resistance genes have been tagged, paving the way for pyramiding of diverse resistance genes for durable gall midge resistance.

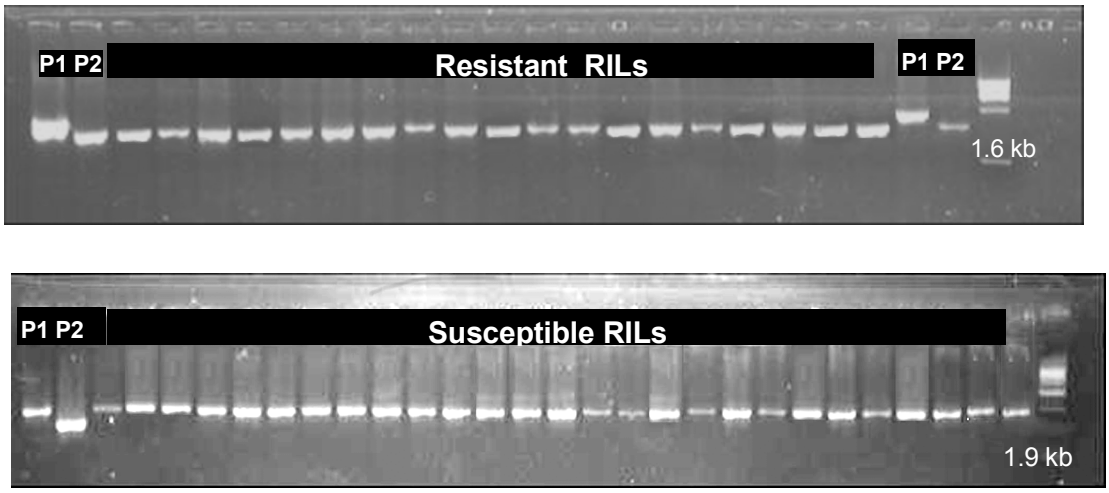


Fig. 1 Genotyping of $F_8$ population derived from the cross TN1 X RP 2068 using a functional marker, gm3NBSLRR5. A 1.6 kb amplicon is specific for the resistance allele and 1.9 kb for the susceptible. No recombinants were observed between marker genotype and trait phenotype (n=300). P1-TN1, P2-RP2068.

**References**

Bentur J. S., P. Vijayalakshmi, V. S. A. K. Sama, Ch. Padmavathy and K. Himabindu, 2009. Sources of resistance to the new 4M biotype of rice gall midge (*Orseolia oryzae*) Indian J. Agric. Sci. 79: 844-846.

Himabindu K., K. Suneetha, V. S. A. K. Sama, C. Cheralu, P. R. M. Rao and J. S. Bentur, 2009. A new gall midge resistance gene in rice variety MR1523. Rice Genet. Newslett. (in Press).

Jain A., A. Kumar, M. N. Srivastava and S. Nair, 2004. Tagging and mapping of a rice gall midge resistance gene, Gm8, and development of SCARs for use in marker-aided selection and gene pyramiding. Theor. Appl. Genet. 109: 1377-1384.

Katiyar S. K, Y. Tan, B. Huang, G. Chandel, Y. Xu, Y. Zhang, Z. Xie and J. Bennett, 2001. Molecular mapping of gene *Gm-6(t)* which confers resistance against four biotypes of Asian rice gall midge in China. Theor. Appl. Genet. 103: 953-961.

Kumar A., A. Jain, R. K. Sahu, M. N. Shrisvastava, S. Nair and M. Mohan, 2005. Genetic analysis of resistance genes for the rice gall midge in two rice genotypes. Crop Sci. 45: 1631-1635.

Sahu V. N., R. Mishra, B. P. Chaudhary, P. S. Shrivastava and M. N. Shrivastava, 1990. Inheritance of resistance to gall midge in rice. Rice Genet. Newslet. 7: 118-121.

Sundaram R. M. 2007. Fine mapping of rice gall midge resistance genes *Gm1* and *Gm2* and validation of the linked markers. Thesis submitted to Dept. of Plant Sciences, University of Hyderabad, Hyderabad.

Vijaya L. P., S. Amudhan, K. Himabindu, C. Cheralu and J. S. Bentur, 2006. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. Int. J. Insect Sci. 26: 207-211.