

# New Genetic Avenues for Insect Pest Management in Rice as Revealed by Studies on Gall midge

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## Abstract

*Insect pests pose a major threat to rice productivity and production. Globally, a sizeable portion of crop is lost to various kinds of insect pests like stem borer, gall midge, planthopper and leaf feeder. Injury due to planthoppers is compounded with transmission of viral diseases. Among various components of insect pest management, genetic approach through development and deployment of insect resistant elite rice lines is the most ecologically safe and economically attractive proposition. Identification and utilization of natural resistance available in the large primary and secondary gene pools has already resulted in significant economic gains during the last five decades. Large scale and often unplanned deployment of resistant rice cultivar has led to the second generation problems like development of virulent populations of the pest biotypes, capable of overcoming the host resistance and non-target pests attaining importance. Recent advance in molecular biology and availability of biotechnological tools have brought in a paradigm shift in our approach to explore and deploy plant resistance against insect pests towards more rational ways to achieve multiple and durable resistance. In light of our own studies on rice-gall midge interactions and other available reports we try to provide some insights into possible novel ways of exploring plant's genetic resistance.*

**Keywords:** Rice, insect pests, gall midge, genetic resistance, insect virulence gene

## Plant resistance genes

We were the first to map and tag naturally occurring gall midge resistance genes in rice and design molecular markers for the marker-aided selection of several resistance genes found in the rice germplasm (Nair et al 2011). One of the major problems encountered in the development of rice varieties resistant to gall midge is that the resistance is governed by a single gene and over a period of time gall midge develop resistance, through the evolution of new biotypes, against the deployed resistance genes. This renders the resistance gene useless in subsequent efforts to breed resistant rice lines. Characterization of virulence spectrum of gall midge populations across the country led to defining seven distinct biotypes (Vijayalakshmi et al 2006). Likewise, spectrum of resistance conferred by the gall midge resistance helped to categorize these genes into four groups (Table 1). Superimposing on this interaction matrix, the two distinct nature of resistance, HR+ and HR- types, has helped to identify suitable gene combinations like *Gm4+* *Gm8* or *gm3+Gm8* or *Gm1+Gm4* that are likely to confer a wide spectrum and durable resistance. Closely linked molecular markers for

these genes have been developed to undertake such gene pyramids. Partial cloning of the putative candidate gene, *Gm4* (LRR) (Himabindu 2009) and *Gm2/gm3* (NB-ARC) (Sama 2011) and sequencing of alleles from resistant and susceptible genotypes revealed that alleles from the resistant lines were truncated with deletions. Such phenomenon of deletions resulting in expression of resistance phenotype needs further elaboration for understanding evolution of plant resistance genes and discovery of new and novel alleles.

## Molecular basis of resistance

An understanding of the molecular basis of rice-gall midge interactions would provide basic information on nature and durability of plant resistance and insect virulence. Resistance genes differ in the nature of resistance. All known genes except *Gm1* and *Gm8* exhibited resistance accompanied with hypersensitive reaction (HR+ type), while these two genes displayed resistance without the expression of HR (HR-type). We employed various gene expression profiling and validation techniques to study such interactions. It is evident from these studies that HR+ type of resistance is

typically gall midge inducible plant defence as in several plant-pathogen systems (Rawat et al 2010). However, several of the genes like *Gm2*, *gm3* and *Gm4* are known to confer this type of resistance but differ in their spectrum of resistance across gall midge biotypes and in nature of inheritance, either as dominant or as recessive traits. Some of the genes like *gm3* and *Gm4* have been closely mapped within the narrow genomic regions and possible candidate genes have been identified. Cloning and sequencing these genes would help us understand basis of wide spectrum of resistance.

In contrast, HR- type of resistance in rice variety Kavaya having *Gm1* gene displayed a novel mechanism (Rawat et al 2012a). Although this gene does not induce any hypersensitive response to pest attack, it appears to be constitutively expressed. Though closely mapped on chromosome 9, this gene as yet eludes proper identification and elaboration in terms of structure and function. In view of the proven track of durability of this gene, further intensive studies would provide us the basis of durability of resistance. Another known gene, *Gm8*, also expresses HR- type resistance but has wider spectrum of resistance. It is important to know whether resistance conferred by this gene is similar to that by *Gm1* and look for more novel genes with HR- type resistance.

We probed further into the molecular basis of plant susceptibility (Rawat et al 2012b). As could be expected, all the known defense related genes were found to be down regulated during a compatible interaction while set of genes related to primary metabolism, nutrient transport, nutrient relocation, cell organization and DNA synthesis were upregulated.

Development of gall is not a normal part of the plant morphogenesis and hence involves activation of genes unique to this process. Tumour responsive protein coding gene could be one candidate gene involved in the process of gall development. These studies, however, did not bring out key susceptibility genes. More intensive research is suggested to discover such susceptibility genes that are now being explored as novel class of target genes in host-plant resistance. Induction or detection of mutant forms or silencing existing susceptibility genes can confer novel mechanisms of host-plant resistance.

### Insect virulence genes

We also explored the rice gall midge genome to get an insight into insect virulence. Using next-generation sequencing (Roche 454 pyrosequencing) strategy over 80,000 ESTs each from insects feeding on resistant or susceptible host genotypes were identified (Sinha et al 2012). Comparative transcriptome analysis of these two sets of ESTs has identified several key genes involved in insect virulence or avirulence. Additionally, this study generated a huge set of potential EST-based microsatellite markers (2,303) and SNPs (2,756) that can be utilized for biotype differentiation and population biology studies.

Investigating a set of serine proteases as target genes in the insect, we cloned two genes, *OoproI* and *OoproII* and sequenced these (Sinha et al 2011). Quantitative real time PCR analysis revealed that both the genes were significantly upregulated in larvae feeding on resistant cultivar than in those feeding on susceptible cultivar suggesting their role in detoxification of plant resistance factors.

**Table 1. Characteristic reaction patterns of seven gall midge biotypes (GMB) against the set of gene differential rice varieties (Nair et al 2011)**

Group	Designation	Resistance Gene	Nature of resistance	Reaction pattern against gall midge biotypes						
				GM B1	GM B2	GM B3	GM B4	GM B4M	GM B5	GM B6
I	W1263	<i>Gm1</i>	HR-	R	S	R	S	S	R	R
	Phalguna	<i>Gm2</i>	HR+	R	R	S	S	S	S	S
	ARC5984	<i>Gm5</i>	HR+	R	R	S	S	S	S	S
II	Dukong 6	<i>Gm6</i>	HR+	R	R	S	S	S	S	S
	RP2333156-8	<i>Gm7</i>	HR+	R	R	S	S	S	S	S
	Madhuri L9	<i>Gm9</i>	HR+	R	R	S	S	S	S	S
	BG 380-2	<i>Gm10</i>	HR+	R	R	S	S	S	S	S
III	CR57-MR1523	<i>Gm11</i>	HR+	R	R	R	R	S	S	S
IV	RP2068-18-3-5	<i>gm3</i>	HR+	R	R	R	R	R	S	S
	Abhaya	<i>Gm4</i>	HR+	R	R	R	R	R	S	S
	Aganni	<i>Gm8</i>	HR-	R	R	R	R	R	S	S
V	TN1	None	--	S	S	S	S	S	S	S

Another gene oligosaccharyltransferase (*OoOST*), coding for one of the secretory salivary proteins, was also cloned and characterized (Sinha et al 2011b). Tissue-specific analysis of the expression of *OoOST* revealed over 7-fold increase in the transcripts of the gene in salivary glands of maggots feeding in susceptible plants than in those feeding in resistant hosts, thus implicating their role in insect virulence. Intensification of research along these lines will lead to identification more genes and pathways involved in insect virulence and evolution of biotypes. Such studies will also help to decipher insect interaction in greater detail and eventually lead to novel genetic strategies for insect pest management in rice.

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