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MOLECULAR DISSECTION OF BIOTYPE EVOLUTION IN RICE GALL MIDGE (*ORSEOLIA ORYZAE*): INTEGRATING CLASSICAL EVIDENCE WITH MULTI-OMICS INSIGHTS FROM TELANGANA AND INDIA

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ABSTRACT
Rice gall midge, *Orseolia oryzae* (Wood-Mason, 1889), is an important insect pest of rice in India, which is highly evolutionarily adaptable to the long-term selection pressure of its host plants. A brief summary of the historical emergence, biotype diversification, molecular evolution and resistance breeding of rice gall midge populations in India is provided. Early research found that these biotypes could be separated in different reaction patterns to the resistant rice varieties that contain rice gall midge resistance genes (*Gm*). The repeated occurrence of virulent biotypes (e.g. GMB4, GMB5, GMB6 and GMB4M) indicated an increasing virulence spectrum and also indicated a rapid breakdown of resistance under high levels of cultivation of resistant cultivars. A large number of germplasm screening programmes found a number of important resistance donors, including Eswarakora, PTB-series lines, Siam 29, Leuang 152 and wild *Oryza* species and major resistance genes were identified, including *Gm1, Gm2, Gm3, Gm4* and *Gm8*. Both RAPD and ISSR molecular analysis, SSR, mitochondrial DNA and SNP-based analysis showed a significant amount of genetic divergence, phylogeographic structuring and localized adaptation among the populations of Indian gall midges. In addition, transcriptomic and effector biology studies further showed that virulent populations regulate host defence pathways by detoxification genes and stress-response proteins and defence suppressors. It was emphasized that interaction between rice gall midge (RGM) and rice was an arms race which needs to involve multiple resistance sources, molecular surveillance, gene pyramiding and genomic breeding strategies to achieve durable and sustainable management of RGM in changing agro-climatic conditions.

KEYWORDS: Rice gall midge, Biotype, *Gm* genes, Multi-omics, Host–insect interactions, Virulence adaptation, Evolutionary genomics

1. INTRODUCTION

Rice is one of the main cereal crops in Asia and an important source of food security in India with more than half of the country's cereal production coming from rice (Kakde *et al.*, 2014). It is however, greatly limited by insect pests which cause almost 25% of the biotic yield loss (Yarasi *et al.*, 2008). Of these, the Asian rice gall midge, *O. oryzae* (Wood-Mason, 1889) is considered to be especially devastating as it causes silver shoots resulting in failure of panicle formation and a significant yield loss (Muralidharan & Pasalu, 2005; Krishnaiah & Varma, 2011). One of the areas of gall midge control that is difficult is its ability to develop virulent biotypes which can outcompete host resistance. Early studies based on differential host reactions established the existence of multiple biotypes (Roy *et al.*, 1971; Kalode & Bentur, 1989; Bentur *et al.*, 2003). Numerous attempts of widespread propagation of resistant cultivars have, however, resulted in breakdown of resistance and the development of new biotypes, such as GMB4, GMB5 and GMB6, across various agro-ecological zones (Bentur *et al.*, 1987; Nair & Devi, 1994; Singh & Chaudhary, 1996). These patterns are dynamic in nature and are evolutionary in result, being the outcome of a selection process. In recent years, the development of molecular biology techniques has contributed to the understanding of the mechanisms of virulence and adaptation. Genomics and transcriptomics studies have shown how differential gene expression, effector proteins and metabolic pathways are involved in the host–insect interactions (Rawat *et al.*, 2012; Sinha *et al.*, 2012; Nandana *et al.*, 2025). Complementing these molecular insights with traditional biotype characterization is crucial for the understanding of the evolution of gall midges and for the development of durable resistance strategies.

2. Ontogeny and Ecology of Rice Gall Midge

The life cycle of *O. oryzae* takes 3-4 weeks in favourable condition (Krishnaiah and Varma, 2011). Adults are small, delicate, mosquito-like flies (3.0-3.5 mm) which the females lay 100-300 eggs singly or in small clusters around the ligule area of the leaf blades or sheaths (Bentur *et al.*, 2003). Eggs hatch within 3-4 days and give rise to apodous larvae which move towards the apical meristem but do not penetrate the tissue directly (Sinha *et al.*, 2012). The larval stage lasts for 15-20 days and is the most destructive stage in the development of the insect. Larvae feed and causes the gall formation (silver shoot) by modifying the differentiation of host tissues and inhibit normal tiller growth (Rawat *et al.*, 2012). The pupation takes place in the gall and lasts for 2-8 days, before the emergence of the adults, the pupa is partially exposed (Muralidharan and Pasalu, 2005). The whole immature growth occurs on a single tiller, providing the protection and efficient use of host resources (Bentur *et al.*, 2011). Under ecological consideration the pest is well established under humid condition in irrigated and rainfed low land rice ecosystems with high tillering stage of the active crop (Krishnaiah and Varma, 2011). High humidity and continuous cultivation of rice coupled with the delay in sowing are conducive for infestation (Rathod *et al.*, 2021). Population peaks are generally found during late August to early October in monsoon conditions (Pasalu and Katti, 2006). Alternate hosts, such as *Leersia hexandra, Echinochloa crusgalli* and wild rice species such as *Oryza* nivara and *Oryza* rufipogon are sources of population carryover during the off-season (Nair *et al.*, 1996; Bentur *et al.*, 2003). There is no sexual dimorphism variation related to virulence variation (Kalode and Bentur, 1989; Bentur *et al.*, 2003) and no any variation observed in the morphology of *O. oryzae* in all the biotypes (GMB1-GMB6). The males are smaller (3.0 mm), slender and have long antennae (23 segments) while females are larger (3.5–4.0 mm) and have fewer antennal segments (13) (Sinha *et al.*, 2012). Males have a shorter lifespan and they have multiple mating, whereas females have a longer lifespan and generally mate only once before they oviposit (Sinha *et al.*, 2012). Conserved traits suggest that the biotype variation is not due to morphology, but rather to genetic and physiological traits (Bentur *et al.*, 2011; Nair *et al.*, 1996).

3. Endemic Distribution and Ecological Hotspots

Gall midge is found throughout the Asian rice eco-region and India is known as a centre of endemism under different agro-climatic situations (Bentur *et al.*, 2003) (Figure 1). It is well established that the pest is mainly linked to irrigated and rainfed lowland ecosystems with high RH and continuous availability of host plants which maintain endemic populations of the pest (Krishnaiah and Varma, 2011). Longer duration of the crop and intensive cultivation further boosts the pest persistence and outbreak potential (Rathod *et al.*, 2021). Some other ecological evaluations have highlighted the importance of cropping intensity and favourable microclimate for maintaining pests (Sarao *et al.*, 2021). Different ecological hotspots are observed all over eastern, central and southern parts of India which are mainly controlled by the climatic stability and cropping intensity (Kalode and Bentur, 1989). The east (Odisha, West Bengal, Bihar, Jharkhand and eastern Uttar Pradesh) is prone to recurrent outbreaks, with conducive conditions for the spread of these outbreaks—monsoon dominated conditions and extensive lowland rice cultivation (Dash *et al.*, 2021). In the central zone of the country (Chhattisgarh and Madhya Pradesh), the population is stable with non-simultaneous sowing in humid environment, which allows local adaptation and slow diversification of biotype (Kumar *et al.* 2020). The southern peninsular region, especially Andhra Pradesh, Telangana, Karnataka, Tamil Nadu and Kerala, is a hotspot of virulence shifts and biotype evolution with high number of multi-season cropping system and irrigated command areas (Bentur *et al.*, 2011). Infestation is further exacerbated under consistently humid and favourable microclimatic conditions (Cheng *et al.*, 2021) in coastal ecosystems. There have also been recent studies that have shown an ongoing adaptation in virulence patterns across southern India (Vijaykumar *et al.*, 2022).

The Telangana state is home to the gall midge as a stable endemic pest in the major rice growing areas like Warangal, Karimnagar, Khammam and Nizamabad (Vijaya Lakshmi *et al.*, 2006). The pest is most prevalent during the monsoon and late kharif seasons, due to high humidity and late transplanting which facilitates the quick population build-up of the pest (Nair and Devi, 1994). This ensures uninterrupted host availability as continuous cultivation of rice and overlapping stages of cultivation further contribute to this (Sarao and Bentur, 2016). Irrigated command areas are important areas to support the persistence of pests throughout the entire year and high pest pressure (Krishnaiah and Varma, 2011). Telangana has been considered as a continuous hotspot for evolution of new virulent biotypes (Bentur *et al.*, 2011) and the continuous deployment of resistant cultivars offers an evolutionary selection pressure that eventually stabilizes and introduces changes in virulent biotypes. Alternate host plants include *L. hexandra, E. crusgalli*, *O. nivara* and *O. rufipogon* that provide reservoirs for population carry over (Nair *et al.*, 1996). The findings from the recent field investigations in different parts of Telangana and the adjoining areas also reiterate the persistence of pests and their adaptive variability under the local conditions (Murali Krishna *et al.*, 2024). Host resistance and pest dynamics are also assessed in contemporary ways, which support long-term ecological adaptation and persistence under varying climate scenarios (Hitesh Kumar *et al.*, 2025).

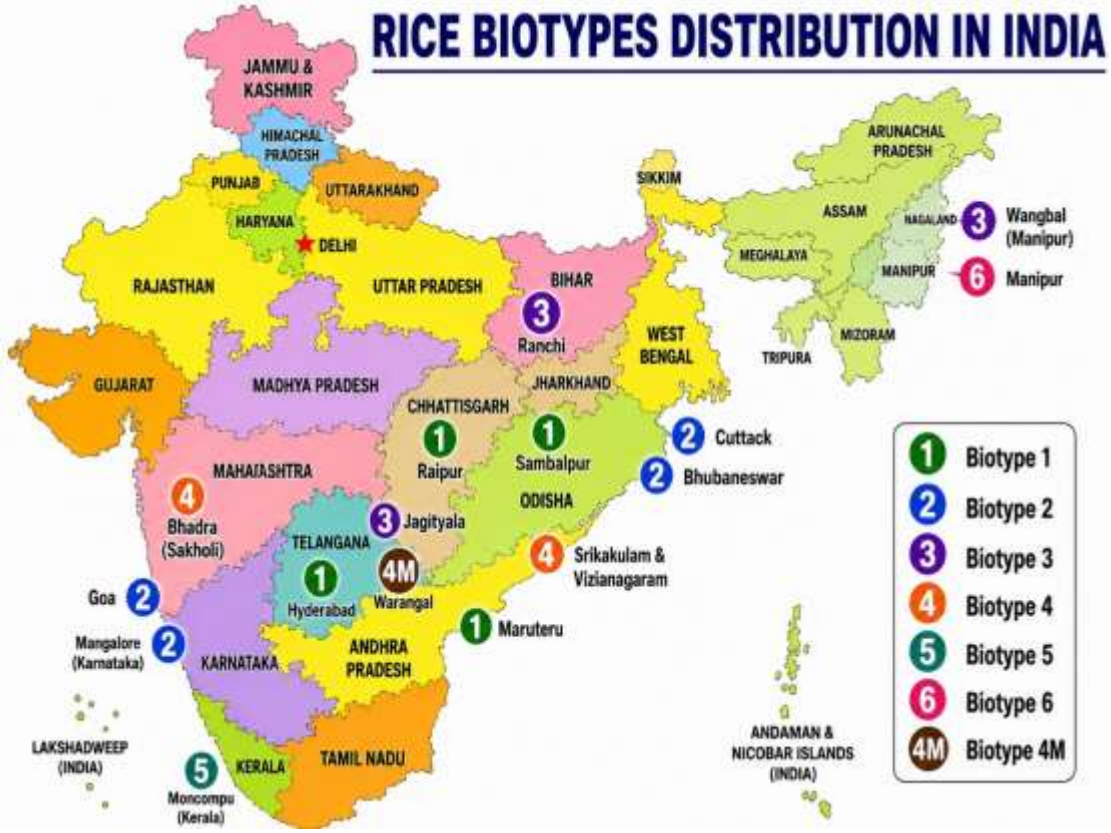


Figure 1. Distribution of Rice Gall Midge (*Orseolia Oryzae*) in India

4. Definitions and Concept of Biotypes in Rice Gall Midge

According to Heinrichs *et al.*, (1985) a biotype is a genetically differentiated population of a species which shows variability in its capacity to survive, develop and reproduce on a given host genotype. The presence of gall midge biotypes in rice has been reported based on differential reactions on rice cultivars with distinct resistance genes (Israel and Vedamoorty, 1953; Khan & Murthy, 1955; Roy *et al.*, 1971; Pathak and Khan, 1994) which showed the existence of physiologically specialized populations within a morphologically homogeneous species. These populations displayed differential virulence, but failed to show any associated morphological differences, reflecting functional genetic heterogeneity, which is controlled by host-insect compatibility (Kalode and Bentur, 1989; Bentur *et al.*, 2003). These interactions were found to be specific and were explained by Bentur *et al.* (2011) in the gene-for-gene relationship between resistance genes in rice and complementary virulence determinants in the insect population. A detailed study under various agro-ecological conditions and cultivation of resistant varieties in various countries of India revealed the occurrence of virulent biotypes, their persistence and their replacement (Kalode and Bentur, 1989). In endemic regions such as Telangana, there were progressive changes in virulence patterns such as continuous use of resistance genes (Vijaya Lakshmi *et al.*, 2006). Subsequent molecular research found that mutation, recombination, directional selection and localized adaptation played a great role in the evolution of virulence in *O. oryzae* populations (Atray *et al.*, 2015). Further transcriptomic studies showed that there exist effector-associated genes that are differentially expressed in the infection process of gall induction and manipulation of the host (Sinha *et al.*, 2012); population genetic studies also demonstrated that in geographically structured populations, there exist genetically differentiated virulent groups (Bentur *et al.*, 2011). Recent research work in the field and molecular has revealed ongoing biotype variability, spatial dominance of virulent biotypes and changes in the biotype composition within rice ecosystems (Kumar *et al.*, 2020; Sarao *et al.*, 2021). The effect of climatic variability on seasonal incidence and population dynamics in endemic agro-ecosystems were also affected (Rathod *et al.*, 2021). Recent studies showed progressive evolutionary diversification and spatial restructuring of rice gall midge biotypes in intensified rice production systems, as a result of the selection pressure exerted by the rice resistance genes, along with the ecological heterogeneity and the dynamics of adaptive virulence (Vijaykumar *et al.*, 2022; Murali Krishna *et al.*, 2024; Hitesh Kumar *et al.*, 2025). To date, six to seven major biotypes have been identified in India (Bentur *et al.*, 2003; Vijaya Lakshmi *et al.*, 2006) and other virulent biotypes have been reported in various parts of Asia, the rice production zone, further supporting the idea of continuous evolutionary diversification within the species.

5. Classification and Distribution of Rice Gall Midge Biotypes in India

Classification of rice gall midge biotypes in India is primarily based on their differential reactions to rice varieties carrying specific resistance genes. The distribution of these biotypes varies across rice-growing regions, reflecting local adaptation, host selection pressure and ecological diversity.

5.1. Classification Based on Differential Host Reactions: The biotype is a population of a species that is genetically distinct and which shows a differential survival, development and reproductive capacity on genotype(s) of its host.

(Heinrichs *et al.*, 1985) Differential reactions on rice cultivars with different resistance genes have led to the identification of gall midge biotypes; the finding of physiologically specialized populations within a morphologically homogeneous species (Israel and Vedamoorty, 1953; Khan & Murthy, 1955; Roy *et al.*, 1971; Pathak and Khan, 1994). The virulent nature of these populations did not correspond to the morphological variation and these populations were functional genetic heterogeneous and controlled by host-insect compatibility (Kalode and Bentur, 1989; Bentur *et al.*, 2003). Such specificity of the interactions was characterized by the gene-for-gene relationship, which suggested that genes for resistance in rice matched complementary genes for virulence in the insect population (Bentur *et al.*, 2011). Detailed investigations were conducted all over India and virulent biotypes were found to emerge, survive and replace in many agro-ecological environments and when the resistant varieties were cultivated over a long period of time (Kalode and Bentur, 1989). It was observed that there were progressive changes in virulence patterns in endemic region where there was continuous deployment of resistance genes (Vijaya Lakshmi *et al.*, 2006). Further molecular analyses showed that mutation, recombination, and directional selection and local adaptation played major roles in the evolution of virulence among *O. oryzae* populations (Atray *et al.*, 2015). These studies also included transcriptomic analyses of differential gene expression that were linked to the processes of gall formation and host manipulation (Sinha *et al.*, 2012) and population genetic analyses that showed that there were genetically differentiated virulent groups within geographically structured populations (Bentur *et al.*, 2011). A recent field and molecular investigation showed that there remains variability in the breakdown of resistance and local dominance of virulent populations and dynamics in the biotype composition of rice ecosystems (Kumar *et al.*, 2020; Sarao *et al.*, 2021). Also seasonal changes in incidence and population fluctuations in endemic agro-ecosystems were affected by climatic variability (Rathod *et al.*, 2021). Recent studies also showed that as rice cultivation intensified, selection pressure from the rice resistance genes led to progressive evolution, diversification, and spatial restructuring of rice gall midge biotypes, resulting in the emergence of genetically differentiated and highly virulent populations (Vijaykumar *et al.*, 2022; Murali Krishna *et al.*, 2024; Hitesh Kumar *et al.*, 2025). In India, six to seven major biotypes have been reported (Bentur *et al.*, 2003, Vijaya Lakshmi *et al.*, 2006) and several other virulent biotypes have been reported in various rice growing regions of Asia, suggesting on-going evolutionary diversification of the species.

Table 1. Virulence characteristics and resistance gene reactions of major rice gall midge biotypes in India

Biotype	**India**	**Virulence Reaction (Breakdown of Resistance Genes)**	**Avirulent Genes (Effective Resistance)**	**Remarks**
Biotype 1	Widely distributed (early populations)	Does not overcome major *Gm* genes	*Gm1, Gm2, Gm3, Gm4, Gm8*	Considered least virulent; baseline population (Kalode & Bentur, 1989)
Biotype 2	Eastern & Central India	Breaks *Gm1*	*Gm2, Gm3, Gm4, Gm8*	Moderate virulence; first level resistance breakdown (Pathak & Khan, 1994)
Biotype 3	Andhra Pradesh, Telangana, Odisha	Breaks *Gm1, Gm2*	*Gm3, Gm4, Gm8*	Expanded virulence spectrum (Bentur *et al.*, 2003)
Biotype 4	South India (AP, Karnataka, TN)	Breaks *Gm1, Gm2, Gm3*	*Gm4, Gm8*	Highly virulent and widely adapted (Bentur *et al.*, 2003)
Biotype 4M	Warangal (Telangana)	Breaks *Gm1–Gm4*	Partial resistance in *Gm8*	Evolved under strong selection pressure (Vijaya Lakshmi *et al.*, 2006)
Biotype 5	Kerala	Breaks multiple genes including *Gm1–Gm4*	Variable response to *Gm8*	Region-specific adaptation under humid ecosystem (Bentur *et al.*, 2003)
Biotype 6	Manipur (NE India)	Broad virulence including *Gm1–Gm5*	Limited resistance sources effective	Highly specialized NE population (Sarao *et al.*, 2021)
Emerging Biotypes	Karnataka, Telangana, Odisha	Breakdown of *Gm4, Gm8* reported	Limited durable resistance sources remain effective	Indicates ongoing evolution (Vijaykumar *et al.*, 2022; Shravan Kumar *et al.*, 2024)

Figure 2. Heat map showing virulence reactions of major rice gall midge biotypes against differential Gm resistance genes in rice

5.2. Classification Based on Spatial Biotypes: The *O. oryzae* biotypes formed in response to agro-climatic variations and local ecological selection pressures (Kalode & Bentur, 1989). The geographically differentiated populations exhibited different virulence pattern and their compatibility with rice cultivars with different resistance gene (Krishnaiah and Varma, 2011) (Table 2). The humid coastal rice ecosystems of Kerala were mainly dominated by biotype 5 whereas biotype 6 was mainly found in the northeastern hill ecosystems of Manipur (Bentur *et al.*, 2003; Sarao *et al.*, 2021) (Figure 1). A significant level of regional variation in virulence composition was also found across Andhra Pradesh, Telangana, Odisha and West Bengal suggesting high geographical differentiation of different endemic populations (Vijaya Lakshmi *et al.*, 2006; Dash *et al.*, 2021). The study of the mitochondrial genome revealed high levels of sequence variability in the cox1, cox2, cox3, nad1-6, nad4L, atp6, atp8 and cytb genes, which indicated a high degree of evolutionary divergence between geographically distant populations (Atray *et al.*, 2015). Intraspecific differentiation and regional adaptation were also supported by the variation in the mitochondrial control regions (Atray *et al.*, 2015). Genetic variability between the endemic populations was also observed from the analyses of nuclear ribosomal regions like 18S rRNA, 28S rRNA, ITS1 and ITS2 (Behura, 1999; Behura *et al.*, 2001). Genetic polymorphism and population structuring was consistently observed across the rice growing ecosystems by using molecular marker systems such as RFLP, RAPD, AFLP, ISSR and microsatellites, respectively (Mohan *et al.*, 1997; Bentur *et al.*, 2011). Phylogenetic analyses based on AFLP markers additionally divided the populations of Asian gall midges into two broad clades, known as Katiyar *et al.* (2000) as lineage A and B. The Chinese, the Lao and populations from the North Eastern region of India formed one lineage, while the other lineage comprised populations from Nepal, Sri Lanka and other parts of India (Katiyār *et al.*, 2000). The presence of both lineages in the northeastern part of India suggested evolution was taking place in a transition area, with genetic admixture and continuous virulence diversification (Katiyar *et al.*, 2000).

5.3. Classification Based on Temporal Biotypes: Within the same geographical areas, successive cropping seasons resulted in the emergence of temporal biotypes from the same area as continuous host mediated selection and evolutionary replacement of virulent populations (Bentur *et al.*, 2003). The virulence pattern was observed to undergo rapid change and the emergence of new biotypes along with the replacement of the dominant biotype was observed in these populations (Kalode & Bentur, 1989; Pathak & Khan, 1994) (Table 2). The appearance of biotype 4M in Warangal was a documented instance of time-space adaptation due to long-term use of resistant rice varieties (Vijaya Lakshmi *et al.* 2006). Repeated selection of resistant cultivars also resulted in similar change in the virulence spectrum in coastal Karnataka (Vijaykumar *et al.*, 2022). Transcriptional reprogramming and functional genomic modification were found in gall midge populations and related to temporal virulence evolution by molecular investigations. Genes involved in detoxification, including OoDAD1, cytochrome P450 monooxygenases (CYP6 and CYP9), glutathione-S-transferases, carboxylesterases, were found to be involved in stress tolerance and adaptive host utilization, as well as heat shock proteins (Hsp70 and Hsp90) (Sinha *et al.*, 2012; Sinha *et al.*, 2015). Some effector genes and secretion proteins in the salivary glands were also related to the induction of gall, manipulation of host tissues and mobilization of nutrients in susceptible host tissues (Bentur *et al.*, 2011). The adaptive molecular responses allowed virulent populations to outgrow host resistance, to persist on resistant cultivars and to successfully develop in plant tissues.

Table 2. Comparative characteristics of spatial and temporal biotypes of rice gall midge

Category	Spatial Biotypes	Temporal Biotypes
Definition	Geographically structured populations differing across regions	Populations evolving over time within the same location
Key Drivers	Agro-climatic variability, ecological heterogeneity, host distribution	Continuous selection pressure from resistant varieties
Genetic Nature	Stable genetic divergence	Dynamic genetic and transcriptional changes
Morphology	Morphologically indistinguishable (Grover and Prasad, 1980)	Morphologically indistinguishable (Kalode and Bentur, 1989)
Virulence Pattern	Region-specific virulence	Progressive virulence shifts over time
Stability	Relatively stable within region	Rapidly changing across seasons
Examples	Biotype 5 (Kerala), Biotype 6 (Manipur) (Bentur *et al.*, 2003; Sarao *et al.*, 2021)	Biotype 4M (Warangal) (Vijaya Lakshmi *et al.*, 2006); shifts in Karnataka (Vijaykumar *et al.*, 2022)
Geographical Evidence	Andhra Pradesh, Telangana, Odisha, West Bengal (Dash *et al.*, 2021)	Warangal, Coastal Karnataka (Vijaykumar *et al.*, 2022)
Molecular Markers Used	RFLP, RAPD, AFLP, ISSR, SSR (Behura, 1999; Bentur *et al.*, 2011)	Transcriptomics, gene expression profiling (Sinha *et al.*, 2012)
Mitochondrial Genes	*cox1, cox2, cox3, nad1–nad6, nad4L, atp6, atp8* and *cytb* (Atray *et al.*, 2015)	Minor structural variation; mainly functional changes
Nuclear Markers	*18S rRNA, 28S rRNA, ITS1, ITS2* (Mohan *et al.*, 1997)	Expression-level variation rather than sequence divergence
Detoxification Genes	Regionally stable profiles	*CYP450 (CYP6, CYP9), GST, CarE* upregulated (Sinha *et al.*, 2015)
Effector Genes	Population-specific variation	Salivary gland effectors dynamically expressed (Bentur *et al.*, 2011)
Stress-related Genes	Limited variation	*Hsp70, Hsp90* involved in adaptation (Sinha *et al.*, 2015)
Apoptosis-related Genes	Not prominent	*OoDAD1* involved in virulence (Sinha *et al.*, 2015)
Host Interaction Pathways	Stable interaction patterns	Dynamic regulation of *PAL, CHS, LOX, AOS, ROS* pathways (Sinha *et al.*, 2012)
Resistance Genes Involved	Differential response to *Gm1–Gm11* (Bentur *et al.*, 2011)	Breakdown of *Gm4, Gm8* etc. over time
Evolutionary Mechanism	Geographic isolation + long-term selection	Mutation, recombination, directional selection
Population Structure	Two major genetic groups (AFLP) (Katiyar *et al.*, 2000)	Continuous restructuring within populations
Management Implication	Region-specific resistant varieties	Gene pyramiding, rotation, continuous monitoring

5.4. Classification Based on Host–Insect Molecular Interactions: The interactions with the host-insect indicated broad modulation of plant defence pathways during gall midge infestation (Sinha *et al.*, 2012). During compatible and incompatible interaction, defence-associated pathways, such as phenylpropanoid metabolism by PAL and CHS, jasmonic acid biosynthesis by LOX and AOS, salicylic acid signalling pathways and pathways related to reactive oxygen species (ROS) showed significant alteration (Sinha *et al.*, 2012). In the gene-for-gene hypothesis (Bentur *et al.*, 2011), the interactions of the virulence proteins of gall midge with the resistance genes of rice determine compatibility. During 2021-2025, recent investigations using integrated genomic, ecological and field-based analyses showed that the virulence composition was continually changing. The study of screening resistance found significant differences in the host response and performance of resistance genes across various agro-ecological regions (Shravan Kumar *et al.*, 2024). Population-level studies also revealed significant variability in virulence factors and adaptation to a changing environment (Mahantashivayogayya *et al.*, 2024). Additionally, regional studies endorsed that virulence composition has been constantly changing in response to varying climatic conditions and changing agriculture practices (Hitesh Kumar *et al.*, 2025; Sarao *et al.*, 2021). Spatial biotypes (genetically divergent groups of strains adapted to their geographical locations) differed from temporal biotypes (rapid evolutionary turnover; continuous adaptive improvement under sustained selection pressure from hosts) (Bentur *et al.*, 2011; Sinha *et al.*, 2012). In summary, the results indicated that the population of *O. oryzae* is complex and that complete resistance management strategies involving resistance-gene pyramiding and the strategic use of resistant cultivars are needed to prevent the establishment of large populations of rice gall midge (Sarao *et.al.*,2021).

6. Evolution of Rice Gall Midge Biotypes in India

The evolution of rice gall midge in India is a classic example of adaptive evolution where a continuous host/plant resistance gene/virulence mechanism interaction is at play (Kalode and Bentur, 1989; Bentur *et al.*, 2016). Resistant rice cultivars with varying genes for gall midge resistance (Gm) genes were deployed repeatedly, which created strong directional selection pressure on gall midge populations, leading to progressive diversification of virulence patterns across major rice ecosystems in India. In the course of many decades, relatively homogenous nonvirulent strains have become genetically distinct and geographically specialized virulence complexes that have become resistant to several host resistance genes.

6.1. Pre-1970s (Pre-Evolutionary Phase): Indian gall midge populations were relatively uniform before the emergence of resistant rice cultivars as most rice varieties grown in India were highly susceptible to the insect (Pathak and Khan, 1994). A high incidence of severe outbreaks was noted in irrigated rice ecosystems of Andhra Pradesh, Odisha, Tamil Nadu and West Bengal, especially in humid condition conducive to continuous rice cultivation (Khan and Murthy, 1955). The concept of distinct physiological races or biotypes was not confirmed as there were no differential varieties available to type the virulence (Roy *et al.*, 1971). During this time, traditional cultivars were mostly landraces that were not resistant to gall midges, but were adapted to local conditions. Resistant varieties were not present so host mediated selection pressure was relatively low and gall midge populations were not highly virulent (Kalode and Bentur, 1989). As a result, there was a high prevalence of mono-virulent populations throughout endemic ecosystems. Thus, relatively homogenous avirulent populations prevailed throughout endemic ecosystems. At this time, there were no molecular investigations available, but subsequent mitochondrial and population genetic research indicated that natural genetic variability had already arisen within geographically distinct populations (Atray *et al.*, 2015). The ancestral genetic variations may have been the basis for the later virulent biotypes under resistance-mediated selection pressure.

6.2. 1970s–1980s -Initial Virulence Shift Phase : The existence of intraspecific variability in rice gall midge populations was first suggested by Khan and Murthy (1955), who observed contrasting infestation patterns in Warangal and Cuttack before resistant cultivars were introduced. Roy *et al.* (1971) and Chatterji *et al.* (1975) subsequently confirmed differential varietal reactions in Andhra Pradesh and Odisha, indicating the presence of geographically distinct virulence populations. A major breakthrough in biotype characterization occurred under the All India Coordinated Rice Improvement Programme (AICRIP) using multi-location data collected between 1970 and 1983, Kalode and Bentur (1989) developed a differential-host system based on Eswarakora, Siam 29 and Leuang 152. This enabled classification of the first three gall midge biotypes in India. Biotype 1 showed the reaction pattern *R-R-S* and remained avirulent to resistance derived from Eswarakora and Siam 29. Biotype 2 exhibited the pattern *S-R-S* and specifically overcame Eswarakora-derived resistance. Biotype 3 displayed the pattern *R-S-S* and was virulent against Siam 29-derived resistance. These contrasting reactions demonstrated that gall midge populations possessed distinct virulence determinants capable of overcoming specific host resistance genes. Several resistant varieties and donor lines were extensively cultivated during this phase, including Eswarakora, Siam 29, Leuang 152, Ptb 10, Ptb 18, Ptb 21 and Ptb 33 (Kalode and Bentur, 1989). Most of these genotypes carried early gall midge resistance genes such as *Gm1* and related alleles. Continuous cultivation of these resistant varieties imposed strong selection pressure favoring survival of virulent insect genotypes capable of overcoming host resistance. Kalode and Bentur (1989) proposed that these biotypes evolved independently under localized host-selection pressure rather than through sequential evolution. This provided strong support for the gene-for-gene interaction model between gall midge virulence and rice resistance genes. The evolutionary process was therefore mosaic and region-specific rather than linear. The geographical distribution of these early biotypes further highlighted strong ecological structuring. Biotype 1 predominated in Hyderabad, Warangal, Maruteru, Sambalpur and Raipur, whereas Biotype 2 occurred mainly in Cuttack, Bhubaneswar, Mangalore, Goa and Sakoli. Biotype 3 was largely confined to Ranchi and Wangbal regions. Such discontinuous distribution indicated that local agro-climatic conditions, host genotype deployment and ecological isolation strongly influenced virulence evolution. Although advanced molecular tools were unavailable during this period, later RAPD and mitochondrial analyses demonstrated that these geographically distinct populations possessed substantial genetic differentiation, confirming early divergence of virulence lineages (Bentur *et al.*, 2011; Atray *et al.*, 2015).

6.3. 1980s–2000s-Diversification and Regional Adaptation Phase: Indian gall midges had experienced a rapid diversification between 1980s and early 2000s, with extensive cultivation of different Gm genes on resistant rice varieties. From 1980s to early 2000s, Indian gall midges experienced a rapid diversification due to the extensive cultivation of Gm genes (Gall genes) on resistant rice varieties. Virulence selection and adaptive differentiation went on faster in continuous rice mono-cropping and irrigated ecosystems as compared to others (Nair *et al.*, 2011). A large scale evolutionary change had been noticed in the resistant cultivar Phalguna, in Srikakulam and Vizianagaram districts of Andhra Pradesh when major damage was observed in P.P. Bentur *et al.* (1987). Investigations resulted in the discovery of Biotype 4 that is able to beat the resistance of Eswarakora and the Siam 29 sources. Biotype 4 was more virulent than previous biotypes with selective virulence, in that it had cumulative virulence that allows it to overcome a number of resistance genes at once. During this time several resistant varieties with Gm1-Gm4 and related genes were grown on a large scale such as Phalguna, Surekha and IR36 and other improved breeding lines developed in the coordinated rice improvement programmes (Bentur *et al.*, 2003). The use of these genetically identical cultivars multiple times put a strong directional selection pressure on the population of the gall midges, thereby increasing the virulence spectrum. Biotype 4 was a new biotype, with high adaptive selection pressure likely due to the use of resistant cultivars. In the same year, the Directorate

of Rice Research (DRR, 1998) found similar virulent strains in Bhandara, suggesting the evolutionary adaptation of the strains at endemic ecosystems level. Biotype 5 was further reported from Moncompu of Kerala where it has been reported from time to time in the early nineties, resulting in the identification of this biotype by Nair and Devi (1994). This population was not able to survive on a few improved resistant varieties, but did survive on traditional cultivars, e.g. Velluthacheera and PTB10. The research showed that local cultivation practices and indigenous landraces too have an effect on virulence evolution. Among the other evolutionary events that took place in the northeast during the 1995-1998 period, the change in dominance of the previously predominant Biotype 3 populations with the emergence of Biotype 6 in Manipur (DRR, 1994) is significant. Biotype 6 was found to be virulent in broader agro-climatic conditions in the north-east and was highly fit in the same conditions. A molecular analysis confirmed this diversification phase. A high level of genetic diversity was found between the populations of southern, eastern and north-eastern India as demonstrated by both RAPD and ISSR markers analysis (Bentur *et al.*, 2011). The pattern of clustering of populations suggested limited gene flow and potential high local adaptation. Such results confirmed ecological isolation, regional host selection and independent evolutionary pathways to be the drivers of diversification of the gall midges in India.

6.4. 2000s–Present-Emergence of Multi-Virulent and Resistance-Breaking Phase: Indian gall midges have been undergoing evolution since the early 2000s to become highly virulent and multi-virulent in order to be able to survive over multiple resistance genes (Bentur *et al.*, 2016). Highly adaptive virulence populations were selected as a result of cultivation of resistant rice varieties, intensive monocropping and irrigation-based rice ecosystems. This was a significant hit as 4M came up from Warangal, Telangana in 2006 (Vijayalakshmi *et al.*, 2006). A modified form of GMB4 was developed as biotype 4M which showed different reaction on the standard rice varieties (Sahithi *et al.*, 2018). Despite its resistance to multiple genes *(Gm1, Gm2, Gm3 and Gm4)*, and unstable compatibility against some resistant genotypes, 4M remains a good option for growers in the field. In this phase several resistant cultivars and breeding lines with pyramided resistance genes were developed and deployed such as improved cultivars carrying pyramided resistance genes *(Gm1-Gm8)* (Bentur *et al.*, 2016). But, selection of these resistance sources over a long period of time allowed for gall midge populations to evolve complex virulence mechanisms that are able to resist multiple resistance pathways. Substantial adaptive plasticity was found in these advanced populations by using molecular and transcriptomic studies. Sinha *et al.* (2017) showed that when the maggots form galls, they influence the host defence signaling, hormone regulation and mobilization of nutrients. Densities that are virulent were able to down regulate the expression of defence-related genes and diverted host metabolism towards gall formation. Other mitochondrial genome polymorphisms such as structural divergence, gene rearrangements and tandem repeat polymorphisms were also shown between geographically isolated populations by Atray *et al.* (2015). These results indicated that these virulent populations undergoing continuous molecular evolution and genetic adaptation occurred quickly. The effectors and microRNA profiling also suggested complex molecular interactions which allow virulent populations to overcome host defence responses (Sinha *et al.*, 2017). This molecular plasticity is responsible for the quick loss of resistance for several cultivars which were once resistant. Recent virulence expansion was also due to climate variability. Population dynamics shifted and adaptation to various agro-ecological niches was possible due to changes in the distribution of rainfall, humidity and temperature (Nair *et al.*, 2011). As a result, highly virulent biotype complexes are now widespread in a few rice growing area of India.

6.5. Molecular and Genomic Evidence of Biotype Evolution: Recent advances in molecular biology have significantly improved understanding of gall midge evolution in India. RAPD and ISSR marker analyses conducted by Bentur *et al.* (2011) revealed substantial genetic divergence among populations from southern, eastern and northeastern India. These studies confirmed that geographically isolated populations evolved independently under localized ecological and host-selection pressures. Atray *et al.* (2015) sequenced the complete mitochondrial genome of *Orseolia oryzae* and identified tandem repeat polymorphisms, structural rearrangements and control-region variability associated with geographically distinct populations. Such mitochondrial diversity provided evidence for long-term evolutionary divergence and regional adaptation. Transcriptomic analyses by Sinha *et al.* (2017) revealed that virulent gall midge populations manipulate host defence pathways, auxin signaling, cytokinin metabolism and nutrient mobilization processes during gall formation. Virulent maggots suppressed defence-associated genes while activating pathways favoring gall development and larval survival. Micro-RNA profiling further demonstrated molecular suppression of rice defence responses by virulent populations (Sinha *et al.*, 2017). Differential expression of effector-associated genes suggested that adaptive virulence in gall midge populations is controlled through complex molecular interactions between insect and host genomes. Comparative genomic studies also indicated that virulent populations possess enhanced genetic plasticity enabling rapid adaptation under continuous resistance deployment. These molecular findings collectively established that gall midge evolution in India is genetically regulated, dynamic and continuously influenced by ecological and host-mediated selection pressure.

7. Molecular and Genomic Evidence of Biotype Evolution

Molecular and genomic investigations have demonstrated genetic differentiation among rice gall midge populations from diverse rice-growing regions. Such variations indicate ongoing biotype diversification influenced by ecological factors and host plant resistance.

7.1. Gene-for-Gene Interaction and Molecular Basis of Virulence: Bentur and Kalode (1989) demonstrated that the rice-gall midge interaction follows a classical gene-for-gene mechanism in which specific rice resistance genes correspond to matching virulence determinants in gall midge populations. Major resistance genes including *Gm1* from

Eswarakora, *Gm2* from Siam 29 and later *Gm3*, *Gm4*, *Gm8* and *Gm11* were identified through coordinated breeding programmes (Kalode & Bentur, 1989; Bentur *et al.*, 2003). Bentur (1987) reported that Biotype 2 overcame *Gm1*-mediated resistance, whereas Biotype 3 overcame *Gm2*. Subsequently, Bentur *et al.* (1987) demonstrated that Biotype 4 simultaneously overcame both genes, indicating cumulative virulence evolution under continuous host-selection pressure.

7.2. Molecular Marker-Assisted Resistance Breeding and Gene Pyramiding: The application of molecular markers significantly strengthened gall midge resistance breeding programmes in India. SSR markers such as *RM219*, *RM444* and *RM11* along with *STMS* and *SNP*-based markers, were extensively utilized in marker-assisted selection and gene pyramiding programmes (Bentur *et al.*, 2011). ICAR-IIRR and ICAR-NRRI programmes identified marker-linked resistance loci associated with *Gm8* and related resistance genes in Indian rice germplasm (Bentur *et al.*, 2016). Although marker-assisted breeding enhanced resistance deployment efficiency, continuous cultivation of genetically similar resistant varieties intensified selection pressure and accelerated emergence of virulent gall midge populations.

7.3. RAPD and ISSR-Based Genetic Differentiation: RAPD and ISSR marker analyses provided the first molecular evidence for genetic structuring among Indian gall midge populations. Bentur *et al.* (2011) reported high polymorphism and clear clustering among southern and eastern Indian populations, indicating substantial genetic divergence among geographically separated virulence groups. Populations from Warangal and Cuttack exhibited distinct molecular profiles despite comparable ecological adaptation patterns, suggesting independent evolution of similar virulence traits under parallel selection pressure. These findings confirmed that Indian gall midge populations evolved through localized adaptive divergence rather than simple geographical spread.

7.4. Microsatellite (SSR)-Based Population Structuring and Evolutionary Divergence: Microsatellite or SSR-based investigations further clarified population differentiation and regional adaptation among Indian gall midge populations. Bentur *et al.* (2011) observed high allelic diversity and significant FST values among endemic populations, indicating restricted gene flow and localized evolutionary divergence. Biotype 4 populations from Srikakulam and Bhandara exhibited comparable virulence spectra but differed genetically, supporting the concept of parallel evolution rather than direct lineage expansion. These studies established that virulence evolution in gall midge populations is strongly shaped by regional ecological conditions and independent adaptive trajectories.

7.5. Mitochondrial DNA Diversity and Phylogeographic Evolution: Mitochondrial DNA analyses involving *COI* and *COII* regions provided additional evidence for phylogeographic divergence among Indian gall midge populations. Atray *et al.* (2015) identified multiple mitochondrial haplotypes and distinct maternal lineages across endemic regions of India. Biotype 6 populations from Manipur possessed unique haplotypes absent in southern Indian populations, indicating long-term geographical isolation and independent evolutionary divergence, and also reported tandem repeat polymorphisms, structural rearrangements and control-region variability within the mitochondrial genome of *O. oryzae*, confirming substantial regional evolutionary variation.

7.6. SNP-Based Genomic Adaptation and Selection Signatures Associated with Virulence: Recent SNP-based genomic investigations identified adaptive genomic regions associated with virulence evolution and ecological adaptation in gall midge populations. Sinha *et al.* (2017) detected strong selection signatures near detoxification-associated loci, indicating that virulence evolution is driven by adaptive genomic selection rather than random mutational processes. These findings demonstrated that gall midge adaptation under resistant cultivar deployment involves targeted modification of functionally important genomic regions associated with host adaptation and survival.

7.7. Transcriptomic Regulation of Virulence: Transcriptomic investigations substantially improved understanding of molecular mechanisms governing gall midge virulence. Sinha *et al.* (2017) identified differential expression of detoxification-associated genes including cytochrome P450 (*CYP6* and *CYP9*), *glutathione-S-transferases (GSTs)*, *carboxylesterases* and *heat shock proteins* such as *HSP70* and *HSP90* in virulent gall midge populations. Virulent maggots feeding on resistant hosts exhibited strong upregulation of detoxification and stress-response genes, indicating that virulence adaptation is primarily regulated through transcriptional plasticity rather than simple gene acquisition.

7.8. Effector Biology, Defence Suppression and Gall Induction Mechanisms: Effector biology studies revealed that gall midge larvae manipulate host physiology through secretion of salivary gland proteins, defence suppressors and cell wall degrading enzymes (Sinha *et al.*, 2017). These molecules suppress plant immune responses, alter nutrient mobilization pathways and induce gall formation favorable for larval development. Such molecular interactions confirm the existence of a continuous molecular arms race between rice defence pathways and gall midge virulence mechanisms.

7.9. Molecular Evidence of Rapid Adaptive Evolution in Telangana Populations: Molecular evidence from Telangana has provided important insights into rapid adaptive evolution in Indian gall midge populations. Vijaya Lakshmi *et al.* (2006) reported enhanced expression of *cytochrome P450* and *GST genes* in GMB4M populations from Warangal compared with standard Biotype 4 populations, indicating localized molecular adaptation under intensive resistant cultivar deployment. Similarly, Sahithi *et al.* (2018) documented virulence shifts from Biotype 1 to Biotype 3 in Jagtial populations associated with breakdown of *Gm2*-mediated resistance. Population genetic studies further revealed rapid allele fixation, reduced genetic diversity and fine-scale population structuring associated with selective sweeps under

intensive agricultural ecosystems (Bentur *et al.*, 2016). These findings establish Telangana, particularly Warangal, as one of the most important hotspots for real-time evolutionary adaptation in Indian rice gall midge populations.

8. Genetic Resources and Resistance Donors for Rice Gall Midge Management in India

Genetic resources and resistance donors serve as valuable sources of gall midge resistance genes for rice breeding programs in India. These materials facilitate the development of resistant cultivars, reducing pest damage and dependence on chemical insecticides.

8.1. Early Exploration and Identification of Resistance Sources: The identification and exploitation of genetic resistance against rice gall midge has constituted one of the most important components of host plant resistance research in Asian rice ecosystems. Systematic exploration of resistance sources in India commenced during the mid-1950s through coordinated field screening programmes conducted under severe natural infestation at major endemic hotspots including Cuttack and Sambalpur in Odisha and Warangal in Andhra Pradesh (Roy *et al.*, 1969; Kalode and Bentur, 1989). These pioneering investigations established the scientific foundation for resistance breeding against rice gall midge and facilitated identification of several genetically stable donor lines possessing broad-spectrum resistance. Among the earliest and most influential resistance donors identified were Eswarakora, PTB 10, PTB 18, PTB 21, PTB 33, Siam 29, Leuang 152 and Velluthacheera (Roy *et al.*, 1969; Roy *et al.*, 1971). These traditional cultivars and landraces exhibited stable resistance against prevailing gall midge populations and subsequently became indispensable genetic resources in rice improvement programmes throughout India and Southeast Asia (Bentur *et al.*, 2003). Eswarakora emerged as one of the most extensively utilized donors because it carried the major resistance gene *Gm*1, which conferred effective resistance against early gall midge biotypes (Kalode and Bentur, 1989). Similarly, Siam 29 served as the principal donor for *Gm*2-mediated resistance, whereas PTB-series lines from Kerala contributed multiple resistance loci associated with durable and broad-spectrum resistance (Bentur *et al.*, 2016).

8.2. Germplasm Screening and Regional Distribution of Resistance: Field and greenhouse screening of over 60,000 rice germplasm accessions was undertaken for extensive multilocation screening and more than 350 primary resistance donors for the rice gall midge were identified (DRR, 1999) (Figure 3). Many of these accessions resistant to the midge were from the north-eastern Indian states of Assam and Manipur, which implies long-term co-evolutionary interactions between the indigenous rice landraces and geographically structured gall midge populations (Bentur *et al.*, 2003). The strength of association and the continued interaction with the host insects in northeastern India was amazing and indicated that long-term ecological association and continual host-insect interaction led to diversification and stabilization of the resistance alleles in traditional germplasm. Other landraces which are highly resistant and maintain stable resistance under endemic infestation pressure were also obtained from southern Indian states, especially Kerala and Tamil Nadu (Roy *et al.*, 1971). Landraces from these areas had adaptive resistance mechanisms which they had developed over longterm exposure to local gall midge populations. The genetically diverse sources of resistance from geographically structured resistance increased the gene flow for gall midge resistance breeding programmes in India.

8.3. Important Resistance Donors and Their Genetic Significance: The PTB-series donors, particularly PTB 10, PTB 18, PTB 21 and PTB 33 gained their special value in breeding gall midge resistance due to their good performance in different endemic areas and under different biotype conditions (Kalode and Bentur, 1989). The landraces not only provided the major resistance genes but also the polygenetic components related to resistance durability and ecological adaptability (Bentur *et al.*, 2003). International donors like Siam 29 and Leuang 152 contributed to the diversity of Indian resistance breeding programmes by providing new resistance alleles from the germplasm of the South East Asian countries (Pathak and Khan, 1994). Donors like Eswarakora, Pta b33 and Siam 29 were later used in a number of breeding programmes due to their effective resistance to different biotypes of early gall midge found in Indian rice growing regions. From these donor lines and breeding materials, several major gall midge resistance genes (Gm1, Gm2, Gm3, Gm4, gm5, gm6, Gm7, Gm8, Gm9, Gm10 and Gm11) were identified (Bentur *et al.*, 2016). Later on, these genes became valuable targets for marker-assisted introgression and molecular characterization/resistance gene mapping programmes.

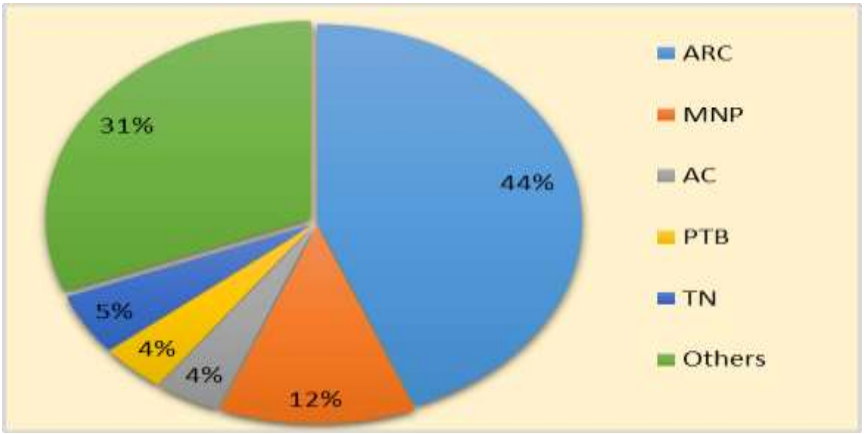
8.4. Expansion of Resistance Screening Programmes: A major expansion of resistance screening programmes occurred during 1993-1997 through a coordinated multi-location evaluation network organized by the Directorate of Rice Research (DRR, 1999). Approximately 13,500 additional germplasm accessions, primarily collected from Madhya Pradesh and adjoining central Indian regions, were systematically evaluated across endemic hotspots under natural infestation conditions (DRR, 1999). These investigations resulted in identification of several novel resistance donors possessing diverse resistance spectra and significantly broadened the genetic reservoir available for resistance breeding programmes (Bentur *et al.*, 2016). The incorporation of newly identified resistance sources enhanced the scope for development of region-specific resistant cultivars with improved adaptability and resistance stability. These programmes also demonstrated the importance of continuous germplasm evaluation because emergence of new virulent biotypes frequently rendered previously resistant varieties vulnerable under changing ecological conditions.

8.5. Development of Secondary Resistance Donors and Resistant Varieties: In addition to primary resistance donors, several secondary resistance sources were developed through strategic intercrossing of resistant parents and utilization of advanced breeding derivatives (Roy *et al.*, 1971). Cultivars such as Phalguna, Surekha, Kavya, Abhaya, RPW 6-13, RPW 6-17 and several MTU derivatives were developed using combinations of resistance genes introgressed from traditional

donor parents (Bentur *et al.*, 2003). These secondary donors possessed improved agronomic attributes including high yield potential, semi-dwarf plant architecture, superior grain quality and wider agro-climatic adaptability while retaining effective resistance against prevailing gall midge populations (Bentur *et al.*, 2016). However, prolonged large-scale cultivation of genetically uniform resistant cultivars subsequently imposed strong directional selection pressure on gall midge populations, leading to emergence of virulent biotypes capable of overcoming previously effective resistance genes (Bentur *et al.*, 2003). The breakdown of resistance in cultivars such as Phalguna later contributed significantly to understanding of biotype evolution and host–insect co-evolutionary dynamics in Indian rice ecosystems.

8.6. Wild *Oryza* Species as Reservoirs of Novel Resistance Genes: Wild relatives of cultivated rice constitute highly valuable reservoirs of gall midge resistance genes. Several wild *Oryza* species including *Oryza brachyantha*, *O. coarctata (Porteresia coarctata), O. eichingeri, O. granulata* and *O. ridleyi* were reported to exhibit high levels of resistance against gall midge infestation (Roy *et al.*, 1971; Bentur *et al.*, 2003). These wild taxa possess unique allelic diversity absent in cultivated rice germplasm and therefore represent important sources of novel resistance genes for future breeding programmes (Bentur *et al.*, 2016). Introgression of resistance loci from wild *Oryza* species through pre-breeding and molecular breeding approaches has gained increasing importance because of the need to develop broad-spectrum and evolutionarily durable resistance against rapidly adapting gall midge biotypes. Wild species-derived resistance is particularly valuable because such genes are often associated with novel defence pathways and reduced vulnerability to existing virulence mechanisms.

8.7. Molecular Characterization and Marker-Assisted Resistance Breeding: Molecular characterization of resistance donors significantly strengthened gall midge resistance breeding in India. Linkage analyses and marker-assisted studies identified several resistance-linked SSR and STMS markers associated with major *Gm* genes including *Gm1, Gm2, Gm4* and *Gm8* (Bentur *et al.*, 2011) (Table 3). Molecular markers such as *RM219, RM444, RM547* and *RM11* were extensively utilized in marker-assisted introgression and pyramiding programmes. Marker-assisted selection enabled development of advanced resistant breeding lines possessing multiple resistance loci and enhanced durability against diverse gall midge populations (Bentur *et al.*, 2016). Nevertheless, recurrent resistance breakdown in several widely cultivated varieties clearly demonstrated the dynamic co-evolutionary relationship between host resistance genes and gall midge virulence mechanisms. The integration of genomics, molecular markers and resistance gene pyramiding therefore represents an essential strategy for developing durable gall midge resistant rice cultivars under rapidly evolving pest populations and changing agro-climatic conditions.



ARC = Assam Rice Collection, MNP = Manipur, TN = Tamil Nadu, PTB = Patambi (Kerala), AC = Orissa
Figure 3. Relative contribution of different rice germplasm collections

Table 3. Catalogued Gall Midge Resistance Genes and Their Corresponding Rice Sources

Gene	Source variety/germplasm	References
Gm1	Usha Samridhi Eswarakora W1263 R30-300 Kakatiya	Chaudhary *et al.* (1985) Chaudhary *et al.* (1985) Reddy *et al.* (1997) Reddy *et al.* (1997) Kumar *et al.* (1998) Motiramani *et al.* (1999)
Gm2	Surekha IET6286 Calrose 70 Siam29, Phalguna CR410-3225	Chaudhary *et al.* (1985) Chaudhary *et al.* (1985) Shrivastava (1998) Mohan *et al.* (1994) Motiramani *et al.* (1999)
gm3	RP2068-18-3-5(IET9244), (Swarnadhan/Velluthacheera)	Kumar *et al.* (1998)

Gm4	Abhaya Balam MNP76	Shrivastava *et al.* (1993) Shrivastava (1992) Shrivastava (1992)
Gm5	ARC5984	Kumar *et al.* (1998)
Gm6	Duokong #1	Tan *et al.* (1993)
Gm7	RP2333-156-8 (IET13404), (Ratna/ARC10659)	Kumar *et al.* (1999)
Gm8	Jhitpiti Aganni	Kumar *et al.* (2000) Sama *et al.* (2012)
Gm9	Line 9 (sister selection of Madhuri, Jaya/Dubraj)	Shrivastava *et al.* (2003)
Gm10	BG380-2	Kumar *et al.* (2005)
Gm11	CR57-MR1523	Himabindu *et al.* (2010)
gm12	MN62M	Leelagud *et al.* (2020)

9. Telangana: A Micro-Evolutionary Epicenter of Gall Midge Dynamics

The Telangana region, particularly Warangal, represents a highly dynamic evolutionary landscape for rice gall midge populations. Initial classifications by Kalode and Bentur (1989) placed populations in Warangal and Hyderabad within Biotype 1, reflecting relatively stable host–pest equilibrium. In Jagtial, a transition from Biotype 1 to Biotype 3 indicates targeted breakdown of Siam 29-derived resistance. In Warangal, further evolution from Biotype 4 to Biotype 4M was documented by Vijaya Lakshmi *et al.* (2006) (Figure 2). The emergence of Biotype 4M signifies fine-scale adaptive divergence, likely driven by continuous exposure to stacked or repeated resistance genes, high cropping intensity and year-round host availability and localized ecological filtering and reduced gene flow. Compared to Kerala (Biotype 5), where adaptation is linked to traditional cultivars, Telangana exemplifies intensification-driven evolution, where modern agricultural practices accelerate pest adaptation. The cumulative evidence across regions supports a multi-phase evolutionary framework as fallow in Table 4.

Table 4. Multi-phase evolutionary framework in India and Telangana

Phase	**Dominant Biotypes**	**Evolutionary Driver**	**Nature of Adaptation**
Pre-resistance era	Implicit variation	Natural diversity	Latent heterogeneity
Differential phase	1, 2, 3	Host resistance genes	Divergent evolution
Resistance breakdown	4	Monoculture pressure	Directional selection
Regional specialization	5, 6	Local cultivar ecology	Adaptive radiation
Telangana phase	4M	Intensive cultivation	Micro-evolution

10. Present Status and Future Evolutionary Trends in India

The Indian rice gall midge is now known to be a collection of dynamic biotypes rather than the stable physiological races; this results from the fact that the former can be found in distinct geographical areas, whereas the latter are ubiquitous throughout India (Bentur *et al.*, 2016). A large number of different populations have evolved which are not all equally compatible with the different Gm resistance genes. The eastern part of India, especially Odisha and West Bengal, southern part of India (Telangana, Andhra Pradesh, Tamil Nadu and Karnataka) and north eastern part of India (Manipur) are still the important areas of gall midge evolution (Kalode and Bentur, 1989). The Warangal region has become significant from the temporal shift perspective as seen in 4M populations in the region in Telangana (Sahithi *et al.*, 2018). Pyramid rice cultivars with multiple resistance genes (Gm1, Gm4, Gm8 and others) are now a focus of breeding programs, and are being developed by marker-assisted selection and genomic breeding techniques (Bentur *et al.*, 2016). But, multi-virulent populations continue to emerge suggesting that one-gene resistance may not be enough to give long-term protection. The future scenarios for evolution will probably include additional virulence complex diversification in combination with climate variation and ecological intensification and constant deployment of resistance. Incorporation of genomics, transcriptomics, effector biology, and molecular diagnostics and ecological surveillance will therefore be critical to predicting future changes in virulence. Ultimately, sustainable gall midge management in India will rely on chemical-resistant breeding and gene pyramiding, molecular surveillance and region-specific integrated pest management (IPM) techniques that will help to reduce the evolutionary advantage of virulent gall midge populations. The development of the gall midge biotypes in India is as follows: Susceptible population is subjected to selection pressure from resistant varieties, which gives rise to virulent biotypes which go on diversifying and form biotype complexes (Figure 4).

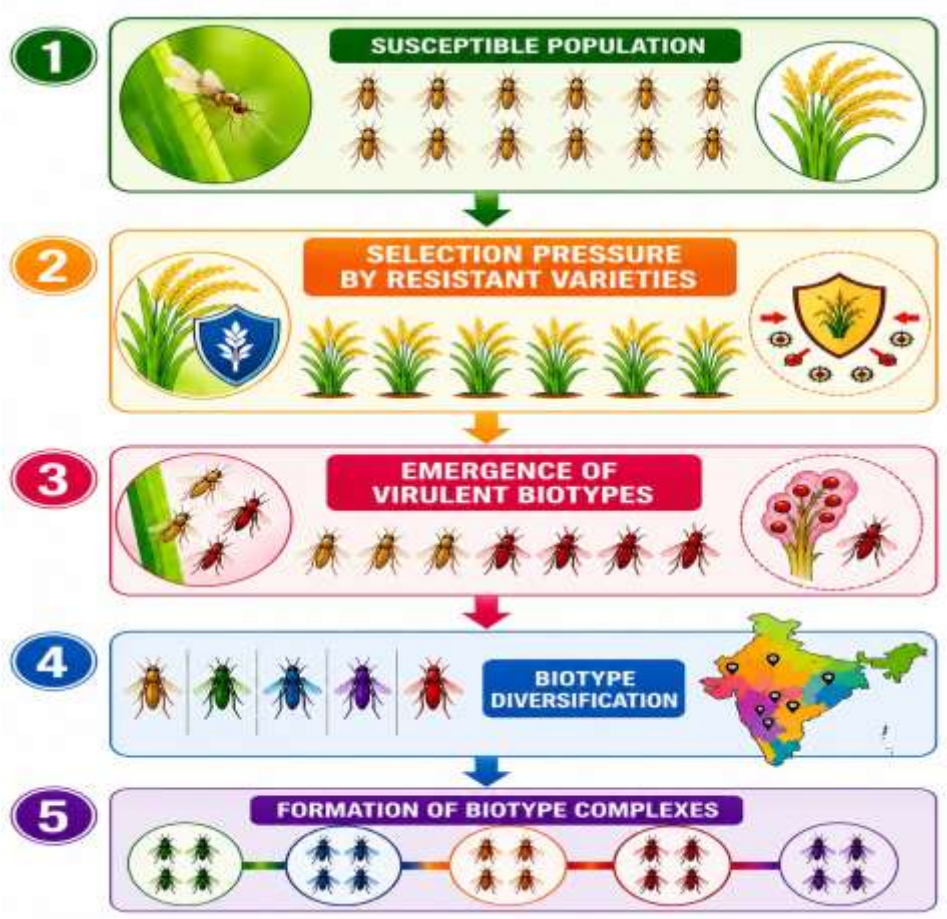


Figure 4. Evolutionary Pathway of Rice Gall Midge

11. CONCLUSION

Rice gall midge (*O. oryzae*) is still one of the most serious issues facing rice and has an extraordinary ability to adapt and develop virulence. It has shown the effect of host resistance deployment, ecological heterogeneity and local selection pressures on pest evolution through the emergence of different biotypes, including highly virulent varieties like 4M, throughout the country. The collection of valuable resistance donors such as Eswarakora, PTB series lines, Siam 29, Leuang 152 and wild *Oryza* species has made significant contributions to the identification of major Gm resistance genes and breeding of resistant cultivars. Molecular investigations have shown that there is significant genetic variation, population structuring and adaptive variability within gall midge populations, and transcriptomic studies have identified a combination of detoxification, stress-response and effector-related genes that were found to be associated with resistance. These results prove that the rice–gall midge system is a co-evolutionary dynamic wherein constant adaptation on both sides is occurring. Continued surveillance of biotype evolution and virulence dynamics will be essential to maintain resistance and for future rice productivity in the face of future agro-climatic changes

REFERENCES

1. Atray, I., Bentur, J. S., & Nair, S. (2015). Complete mitochondrial genome sequence of rice gall midge, *Orseolia oryzae* (Wood-Mason), and comparative phylogenetic analysis. *Mitochondrial DNA, 26*(4), 531–540. https://doi.org/10.3109/19401736.2013.840596
2. Behura, S. K. (1999). Genetic differentiation and molecular characterization of rice gall midge populations in India. *Oryza. 36*(2), 152–158.
3. Behura, S. K., Nair, S., & Bentur, J. S. (2001). Analysis of ribosomal DNA spacer regions in geographically distinct populations of rice gall midge, *Orseolia oryzae*. *Genome, 44*(2), 188–194. https://doi.org/10.1139/g00-109
4. Bentur, J. S. (1987). Virulence adaptation and resistance breakdown in rice gall midge populations of India. *International Rice Research Newsletter, 12*(5), 18–20.
5. Bentur, J. S., Pasalu, I. C., & Kalode, M. B. (1987). Occurrence of a new biotype of the rice gall midge, *Orseolia oryzae* (Wood-Mason), in Andhra Pradesh, India. *International Rice Research Newsletter, 12*(4), 24–25.
6. Bentur, J. S., Pasalu, I. C., Sarma, N. P., Prasada Rao, U., & Mishra, B. (2003). Gall midge resistance in rice. *Directorate of Rice Research Technical Bulletin*, Hyderabad, India.
7. Bentur, J. S., Rawat, N., Divya, D., Sinha, D. K., Agarrwal, R., Atray, I., & Nair, S. (2011). Rice–gall midge interactions: Battle for survival. *Journal of Insect Physiology, 57*(11), 1430–1437. https://doi.org/10.1016/j.jinsphys.2011.07.001
8. Bentur, J. S., Sundaram, R. M., & Jhansi Lakshmi, V. (2016). Biotype evolution and resistance breeding in rice gall midge. *Indian Journal of Plant Protection, 44*(3), 315–326.

9. Chatterji, S. M., Dash, A. N., & Panda, N. (1975). Differential reaction of rice cultivars to gall midge populations in India. *Oryza, 12*(3), 179–183.
10. Chaudhary, R. C., Khush, G. S., & Heinrichs, E. A. (1985). Genetic analysis of resistance to rice gall midge, *Orseolia oryzae* (Wood-Mason), in rice cultivars. *Theoretical and Applied Genetics, 69*(5–6), 467–473. https://doi.org/10.1007/BF00251079
11. Cheng, J. A., Zhu, Z. R., & Xu, H. X. (2021). Ecological adaptation and pest dynamics of rice gall midge in Asian rice ecosystems. *Crop Protection, 143*, 105561. https://doi.org/10.1016/j.cropro.2021.105561
12. Dash, D., Pradhan, S. K., & Bastia, D. N. (2021). Distribution and ecological incidence of rice gall midge in eastern Indian rice ecosystems. *Oryza, 58*(3), 321–328.
13. Directorate of Rice Research (DRR). (1994). *Annual progress report: Rice gall midge investigations*. Directorate of Rice Research, Hyderabad, India.
14. Directorate of Rice Research (DRR). (1998). *Rice gall midge biotype monitoring and virulence studies in India*. Directorate of Rice Research, Hyderabad, India.
15. Directorate of Rice Research (DRR). (1999). *Multilocation evaluation of rice germplasm for gall midge resistance*. Directorate of Rice Research, Hyderabad, India.
16. Grover, P., & Prasad, J. (1980). Biotypes of the rice gall midge, *Orseolia oryzae* (Wood-Mason), in India. Indian Journal of Entomology, 42, 343–347.
17. Heinrichs, E. A., Medrano, F. G., & Rapusas, H. R. (1985). *Genetic evaluation for insect resistance in rice*. International Rice Research Institute, Manila, Philippines.
18. Himabindu, K., Suneetha, K., Sama, V. S. A. K., & Bentur, J. S. (2010). Identification and mapping of a new rice gall midge resistance gene, *Gm11*, in the rice variety CR57-MR1523. *Rice Genetics Newsletter, 25*, 38–40.
19. Hitesh Kumar, R., Ramesh, V., & Bentur, J. S. (2025). Ecological adaptation and host resistance dynamics of rice gall midge under changing climatic conditions. *Journal of Asia-Pacific Entomology, 28*(2), 101245.
20. Israel, P., & Vedamoorthy, G. (1953). Observations on the occurrence and incidence of rice gall midge in India. *Indian Journal of Entomology, 15*(2), 93–102.
21. Kakde, A. M., Patel, K. G., & Chaudhary, P. M. (2014). Insect pest complex of rice ecosystem and its management. *International Journal of Plant Protection, 7*(2), 349–356.
22. Kalode, M. B., & Bentur, J. S. (1989). Characterization and identification of biotypes of the rice gall midge, *Orseolia oryzae* (Wood-Mason). *International Rice Research Newsletter, 14*(6), 5–7.
23. Katiyar, S. K., Bentur, J. S., & Nair, S. (2000). AFLP-based phylogenetic analysis of Asian populations of rice gall midge, *Orseolia oryzae*. *Genome, 43*(6), 1042–1049. https://doi.org/10.1139/g00-070
24. Khan, M. Q., & Murthy, N. S. (1955). Preliminary observations on varietal susceptibility to rice gall midge in India. *Indian Farming, 5*(8), 15–18.
25. Krishnaiah, K., & Varma, N. R. G. (2011). Changing insect pest scenario in rice ecosystem – A national perspective. *Directorate of Rice Research Monograph*, Hyderabad, India.
26. Kumar, A., Bentur, J. S., & Shrivastava, M. N. (2000). Identification of gall midge resistance gene *Gm8* in rice landraces. *International Rice Research Notes, 25*(2), 25–26.
27. Kumar, A., Bentur, J. S., & Shrivastava, M. N. (2005). Identification of a new gall midge resistance gene *Gm10* in rice genotype BG380-2. *Rice Genetics Newsletter, 22*, 56–58.
28. Kumar, A., Shrivastava, M. N., & Bentur, J. S. (1998). Identification of new gall midge resistance genes in rice germplasm. *Oryza, 35*(4), 317–320.
29. Kumar, A., Shrivastava, M. N., & Bentur, J. S. (1999). Inheritance and allelic relationships of gall midge resistance genes in rice. *Oryza, 36*(2), 105–109.
30. Kumar, A., Singh, R., & Patel, D. (2020). Population dynamics and regional adaptation of rice gall midge in central Indian rice ecosystems. *Indian Journal of Entomology, 82*(4), 789–795.
31. Leelagud, P., Bentur, J. S., & Nair, S. (2020). Identification of a novel gall midge resistance gene *gm12* in rice genotype MN62M. *Euphytica, 216*(9), 1–12. https://doi.org/10.1007/s10681-020-02674-9
32. Mahantashivayogayya, K., Reddy, G. P., & Bentur, J. S. (2024). Population diversity and adaptive virulence in rice gall midge under variable ecological conditions. *Indian Journal of Entomological Research, 48*(1), 55–64.
33. Mohan, M., Bentur, J. S., & Nair, S. (1994). Genetic analysis of gall midge resistance in rice cultivar Siam 29 and its derivatives. *Rice Genetics Newsletter, 11*, 119–121.
34. Mohan, M., Nair, S., Bentur, J. S., & Bennett, J. (1997). Molecular marker analysis of genetic polymorphism in rice gall midge populations. *Theoretical and Applied Genetics, 95*(7), 1061–1068. https://doi.org/10.1007/s001220050671
35. Motiramani, N. K., Shrivastava, M. N., & Kumar, A. (1999). Identification and utilization of gall midge resistance genes in Indian rice breeding programmes. *Oryza, 36*(3), 215–220.
36. Murali Krishna, T., Rajasekhar, P., & Praveen Kumar, B. (2024). Field incidence and adaptive variability of rice gall midge populations in Telangana rice ecosystems. *Indian Journal of Agricultural Research, 58*(1), 102–108.
37. Muralidharan, K., & Pasalu, I. C. (2005). Assessments of crop losses in rice ecosystems due to insect pests in India. *Crop Protection, 25*(12), 1354–1360.
38. Nair, N. V., & Devi, K. S. (1994). Identification of a new biotype of rice gall midge in Kerala. *International Rice Research Notes, 19*(1), 19–20.
39. Nair, N. V., Bentur, J. S., & Kumar, P. (1996). Alternate host plants and seasonal survival of rice gall midge, *Orseolia oryzae* (Wood-Mason), in South India. *Oryza, 33*(2), 120–124.

40. Nair, S., Bentur, J. S., & Mohan, M. (2011). Ecological adaptation and molecular evolution of rice gall midge populations in India. *Journal of Insect Science, 11*(1), 1–14.
41. Nandana, G., Bentur, J. S., & Sundaram, R. M. (2025). Transcriptomic and molecular insights into rice gall midge virulence and host adaptation mechanisms. *Journal of Asia-Pacific Entomology, 28*(1), 101–114.
42. Pasalu, I. C., & Katti, G. (2006). Advances in ecofriendly approaches in rice IPM. *Journal of Rice Research, 1*(1), 83–90.Rathod, T. H., Patel, H. N., & Chaudhary, P. M. (2021). Influence of weather parameters on seasonal incidence of rice gall midge, *Orseolia oryzae* (Wood-Mason). *Indian Journal of Entomology, 83*(2), 275–280.
43. Pathak, M. D., & Khan, Z. R. (1994). *Insect pests of rice*. International Rice Research Institute, Manila, Philippines.
44. Rathod, T. H., Patel, H. N., & Chaudhary, P. M. (2021). Influence of weather parameters on seasonal incidence of rice gall midge, *Orseolia oryzae* (Wood-Mason). *Indian Journal of Entomology, 83*(2), 275–280.
45. Rawat, N., Neeraja, C. N., Nair, S., & Bentur, J. S. (2012). Differential expression of defense-related genes in rice during compatible and incompatible interactions with gall midge. *Rice, 5*(1), 1–13. https://doi.org/10.1186/1939-8433-5-32
46. Reddy, C. S., Bentur, J. S., & Kalode, M. B. (1997). Identification of resistance genes against rice gall midge in rice cultivars and breeding lines. *International Rice Research Notes, 22*(1), 18–19.
47. Roy, J. K., Israel, P., & Krishnaiah, K. (1969). Screening of rice germplasm for resistance to rice gall midge under endemic conditions in India. *Oryza, 6*(1), 45–52.
48. Roy, J. K., Israel, P., & Krishnaiah, K. (1971). Differential reaction of rice varieties to gall midge populations in India. *Oryza, 8*(2), 45–52.
49. Sahithi, S., Bentur, J. S., & Sundaram, R. M. (2018). Virulence variation and adaptation of rice gall midge populations in Telangana, India. *Journal of Entomological Research, 42*(3), 345–352.
50. Sama, V. S. A. K., Himabindu, K., Bentur, J. S., & Nair, S. (2012). Characterization and mapping of gall midge resistance gene *Gm8* in rice landrace Aganni. *Molecular Breeding, 30*(3), 1337–1345. https://doi.org/10.1007/s11032-012-9728-5
51. Sarao, P. S., & Bentur, J. S. (2016). Rice gall midge: Biology, ecology and management strategies. *Indian Journal of Plant Protection, 44*(2), 145–154.
52. Sarao, P. S., Kaur, R., & Sharma, A. (2021). Ecological factors influencing endemicity of rice gall midge in Indian rice ecosystems. *Journal of Entomological Research, 45*(1), 67–74.
53. Shravan Kumar, P., Reddy, G. P., & Bentur, J. S. (2024). Emerging virulence patterns and resistance breakdown in rice gall midge populations of South India. *Indian Journal of Agricultural Sciences, 94*(2), 214–221.
54. Shrivastava, M. N. (1992). Identification of resistance sources against rice gall midge in traditional rice cultivars. *Oryza, 29*(4), 251–255.
55. Shrivastava, M. N. (1998). Genetic studies on gall midge resistance in rice cultivar Calrose 70. *Oryza, 35*(1), 45–48.
56. Shrivastava, M. N., Kumar, A. (2003). Identification of a new gall midge resistance gene *Gm9* in rice breeding line derived from Jaya/Dubraj. *Rice Genetics Newsletter, 20*, 61–63.Bentur, J. S., & Kumar, A. (1993). Inheritance of gall midge resistance in rice cultivar Abhaya. *International Rice Research Notes, 18*(5), 14–15.
57. Singh, R., & Chaudhary, R. C. (1996). Emergence and distribution of rice gall midge biotypes in northeastern India. *International Rice Research Notes, 21*(3), 28–29.
58. Sinha, D. K., Atray, I., Bentur, J. S., & Nair, S. (2015). Feeding on resistant rice leads to enhanced expression of defender against apoptotic cell death (OoDAD1) in the Asian rice gall midge. BMC Plant Biology, 15(1), 1–11. https://doi.org/10.1186/s12870-015-0470-2
59. Sinha, D. K., Bentur, J. S., & Nair, S. (2012). Compatible interaction of rice gall midge and rice involves metabolic manipulation and suppression of host defense pathways. *PLoS ONE, 7*(4), e36294. https://doi.org/10.1371/journal.pone.0036294
60. Sinha, D. K., Bentur, J. S., Sundaram, R. M., & Nair, S. (2017). Transcriptomic regulation of virulence and host manipulation in rice gall midge. *Scientific Reports, 7*, 45264. https://doi.org/10.1038/srep45264.
61. Sinha, D. K., Rawat, N., & Bentur, J. S. (2015). Functional genomics of virulence adaptation in rice gall midge populations. *BMC Genomics, 16*, 694. https://doi.org/10.1186/s12864-015-1872-4.
62. Tan, Y. F., Li, X. H., & Heong, K. L. (1993). Occurrence and virulence variation of rice gall midge populations in Asian rice ecosystems. *International Rice Research Notes, 18*(3), 21–23.
63. Tayathum, K., Hongsriphan, N., & Sirisook, P. (2004). Biotypic variation and virulence diversity of rice gall midge in Southeast Asia. *Crop Protection, 23*(7), 611–617. https://doi.org/10.1016/j.cropro.2003.11.012
64. Vijaya Lakshmi, P., Bentur, J. S., & Pasalu, I. C. (2006). Emergence of a modified virulent population of rice gall midge in Warangal region of Andhra Pradesh. *International Rice Research Notes, 31*(2), 20–22.
65. Vijaykumar, L., Reddy, G. P., & Bentur, J. S. (2022). Virulence shifts and adaptive variation in southern Indian populations of rice gall midge. *Journal of Insect Science, 22*(5), 1–9.
66. Yarasi, B., Sadumpati, V., Immanni, C. P., Vudem, D. R., & Khareedu, V. R. (2008). Transgenic rice expressing *Allium sativum* leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. *BMC Plant Biology, 8*, 102.