SURVEY, VARIETAL SCREENING AND MOLECULAR IDENTIFICATION OF ASIAN RICE GALL MIDGE IN Y.S.R DISTRICT OF ANDHRA PRADESH

BY

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B.Sc. (Hons.) Agriculture

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DECLARATION

I, **Mr. M. MURALI KRISHNA**, hereby declare that the thesis entitled **"SURVEY, VARIETAL SCREENING AND MOLECULAR IDENTIFICATION OF ASIAN RICE GALL MIDGE IN Y.S.R DISTRICT OF ANDHRA PRADESH"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original researchwork done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Tirupati.

Date :

M. MURALI KRISHNA

TAM/2021-045

CERTIFICATE

Mr. MURALI KRISHNA has satisfactorily prosecuted the course of research and that thesis entitled **"SURVEY, VARIETAL SCREENING AND MOLECULAR IDENTIFICATION OF ASIAN RICE GALL MIDGE IN Y.S.R DISTRICT OF ANDHRA PRADESH"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE

This is to certify that the thesis entitled **"SURVEY, VARIETAL SCREENING AND MOLECULAR IDENTIFICATION OF ASIAN RICE GALL MIDGE IN Y.S.R DISTRICT OF ANDHRA PRADESH"** submitted in partial fulfillment of the requirements for the degree of **'MASTER OF SCIENCE IN AGRICULTURE'** of the Acharya N.G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Mr. M. MURALI KRISHNA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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M. Murali Krishna. . . .

LIST OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS

$	Dollar
%	Per cent
/	Per
~	Approximate
ANGRAU	Acharya Nayukulu Goginenni Ranga Agricultural University
ANOVA	Analysis of Variance
ARS	Agricultural Research Station
bp	base pairs
cm	centimeter
cM	Centi Morgan
CTAB	Cetyl Tri methyl Ammonium Bromide
DAT	Days After Transplantation
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
et al.,	and other workers
FAO	Food and Agriculture Organization
Fig.	Figure
g	gram
GMB	Gall Midge Biotype
Hcl	Hydrochloric acid
hr	Hour
i.e.,	Which is to say in other words
IIRR	Indian Institute of Rice Research
IRRI	International Rice Research Institute
kb	kilo base
l	Litre
M	Molar
Mega	Molecular Evolutinary Genetics Analysis
$Mgcl_2$	Magnesium Chloride

mha	Million hectare
min	Minute
ml	Milli litre
mM	Milli mole
mt	Million tonne
mtCOI	mitochondrial Cytochrome Oxidase subunit I
Nacl	Sodium Chloride
ng	Nano gram
nm	Nano meter
^{o}C	Degree Centigrade
PCR	Polymerase Chain Reaction
PD	Plant Damage
PPO	Polyphenol oxidase
PSI	Per cent Similarity Index
R	Resistant
RAPD	Random Amplified Polymorphic DNA
RARS	Regional Agricultural Research Station
RGM	Rice Gall Midge
rpm	Revolutions per minute
S	Susceptible
SCAR	Sequence Characterized Amplified Region
SPSS	Statistical Package for Social Sciences
SS	Silver Shoot
SSR	Simple Sequence Repeat
TBE	Tris Borate EDTA (buffer)
TE	Tris EDTA
UV	Ultra Violet
Viz.,	Namely
µg	Micro gram
µl	Micro litre
µM	Micro Mole

ABSTRACT

Name of the Author	:	**M. MURALI KRISHNA**
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The present work on "SURVEY, VARIETAL SCREENING AND MOLECULAR IDENTIFICATION OF ASIAN RICE GALL MIDGE IN Y.S.R DISTRICT OF ANDHRA PRADESH**"** was carried out at Agricultural Research Station, Utukur, Kadapa and Regional Agricultural Research Station, Tirupati, ANGRAU during 2022-23.

In the *kharif* and *rabi* seasons of 2022, a roving survey were conducted in three districts of Southern zone, Andhra Pradesh, for rice gall midge (*Orseolia oryzae*). The highest incidence of silver shoot damage was recorded in Nellore District at 15.38 per cent, while the lowest was observed in Chittoor District at 4.50 per cent.

Studies were conducted at ARS, Utukur, Kadapa to determine the gall midge biotype. The studies used 17 differentials provided by ARS, Ragolu, and 16 differentials supplied by IIRR, Hyderabad. Based on the reaction pattern recorded, it was found that the biotype of Kadapa closely resembled biotype VI. Notably, no damage was observed in W1263, ARC 6605 of group I, ARC 5984 of group II, INRC 3021, and AGANNI of group IV. The per cent similarity index value for Kadapa was determined to be 76.47 per cent.

Studies on genetic variability of gall midge population across Southern zone of Andhra Pradesh, that with the help of SSR, SCAR and mtCOI marker, in that Y132, revealed results that sample populations of Nellore and Chittoor districts belongs to biotype 5 and phylogenetic tree was constructed based on the sequences of mtCOI primers, showed the results as Kadapa (Utukur) sample belongs to biotype 6 (98%) and Nellore and Chittoor District samples belongs to biotype 5 (96%) with separate clusters for each.

In the evaluation of resistance against gall midge, a total of 50 genotypes, including checks, were studied. Among them, two rice genotypes, RPE 937 and RPE 1564, recorded high resistance at both (30 and 50) DATs. These genotypes are considered valuable donors for resistance breeding trials to be conducted at ARS, Utukur, Kadapa. Their strong resistance characteristics make them promising lines for future breeding programs aimed at developing gall midge-resistant rice varieties.

Chapter - I

Introduction

Chapter – I

INTRODUCTION

Rice (*Oryza sativa*. L) is an essential cereal globally and staple diet for half of the population of the world notably in Asian countries. India is the leading producer by area and second leading producer after China. India has 45.1 million ha under cultivation with production of around 121 million tonnes. It contributes 23.5 per cent of global rice production. Within the country, rice occupies one-quarter of the total cropped area, contributing to about 40 to 43 per cent of total food grain production and continues to play a key role in the national food and livelihood security system. Export of rice contributes to nearly 25% of total agricultural exports from the country. However, productivity of rice is only 3.54 metric tonnes/ha of milled rice as against the global average productivity of 3.0 tonnes/ha (FAO, 2020).

In India, West Bengal is the leading rice producing state, followed by Uttar Pradesh, Punjab, Tamil Nadu, Andhra Pradesh. A total of 2.29 million ha rice was under cultivation in Andhra Pradesh with annual production of about 8.64 million tonnes and average productivity of 3.90 tonnes/ha. (www.statista.com 2020-21).

According to research conducted by the Rockfeller Foundation (Herdt, 1991), it was discovered that out of the 20 primary challenges faced in rice production, seven of them are related to biotic factors such as insect pests and diseases. Among these biotic factors, insect pests alone contribute to approximately 10-15 per cent of yield losses, resulting in an estimated overall loss of 21-51 percent in rice production. At the national level, stem borers were responsible for causing 30% of the crop losses. Plant hoppers accounted for 20% of the losses, gall midge caused 15% of the losses, leaf folder contributed to 10% of the losses and the remaining 25% of the losses were attributed to other pests, respectively (Krishnaiah and Varma, 2018).

In India, gall midge pest accounts for an average annual crop loss worth of US $ 80 million (Bentur *et al*., 2003). At present it is considered as major insect pest in India, because of its relative importance.

Upon hatching from the eggs laid on leaf sheath, maggots of rice gall midge crawl down the plant between leaf sheaths to reach the apical meristem. Larval feeding on apical meristem causes transformation of leaf sheath transformed into a tubular gall which is silvery-white in colour and called as silver shoot. The regular tillers transformed into tubular galls, dry off without bearing panicles. Pupae wriggle up along the elongated gall and drill an exit hole near the tip of the gall that allows the emergence of adult fly. The adult midge fly resembles a mosquito. Females are bigger as compared to male with marked difference among two sexes (Nalini and Henry, 1968).

In India, gall midge was recorded from all the rice growing states except Western Uttar Pradesh, Punjab, Uttaranchal, Hill States, Himachal Pradesh, Haryana and Jammu and Kashmir (Bentur *et al.,* 2003).

As per the intensity and regularity of occurrence, few areas have been identified as hot spots for rice gall midge. These are coastal and northern Telangana regions of Andhra Pradesh, Ranchi areas of Jharkhand, entire Chhattisgarh, coastal and Sambalpur areas of Orissa and Tamil Nadu (Mathur and Krishnaiah, 2004).

Lingaraj *et. al.* (2008) reported the incidence of the rice gall midge for the first time during 2004 in Southern districts of Karnataka *viz.*, Kodagu, Mysore and Hassan and the level of infestation in these locations varied between 10-15 per cent.

Prasad (2011), reported that gall midge is prevalent in certain parts of Ranchi, Lohardagga, Khunti, Gumla and Simdega. These places were endemic to the pest where in the damage due to this pest normally ranged from 10 to 70 per cent under congenial climate with an annual yield loss of about 20-70 per cent.

After the widespread cultivation of high yielding gall midge-resistant rice varieties in farmer fields, different populations or biotypes were observed (Singh, 1996). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is the cause for concern. So far, seven biotypes (GMB1 to GMB6 and GMB4M) of gall midge and 11 gall midge resistance genes (Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9, Gm10 and Gm11) have been identified (Vijaya Lakshmi *et al.,* 2006 and Himabindu *et al*., 2010).

Identification of prevailing rice gall midge populations in a particular area is imperative in resistance breeding programmes. If a new biotype of gall midge develops resistance to all known resistant genes *viz., Gm1, Gm2, gm3, Gm4, Gm5* and *Gm6* making entire gene pool susceptible, then it will create havoc to rice cultivation. In this background, there is an urgent need to generate knowledge on identification of gall midge biotypes that are area specific, inheritance and the mode of action of newly identified resistance sources so that genes can be appropriately utilised in resistant breeding programmes (Singh, 2012).

Management practices like cultural, biological, use of resistant varieties, chemical methods may be used to reduce the incidence of gall midge. Farmers habituated to spray chemical insecticides to control insect pests. Since gall midge is an internal feeder, use of insecticides may not be effective. The superior strategy to manage the damage by gall midge in rice is to develop new varieties with high resistance to rice gall midge (Thippeswamy *et al*., 2014).

Chemical management of gall midge is neither very effective nor environmentally safe as the granular pesticides, which are often applied after the pest has exceeded the threshold level did not suffice the yield loss. Consequently, control of the pest is inadequate resulting in the escalation of cost of cultivation (Harathi, 2019).

Since 1970, more than 56 high yielding gall midge resistant rice varieties having different genes for resistance have been released for commercial

cultivation (Bentur *et al*., 2003). Gall midge being endophytic, breeding resistant rice varieties has been a viable and ecologically acceptable approach for management of this pest (Heinrichs and Pathak, 1981).

Identification of new sources of resistance to major insect pests is the prime objective of host plant resistance which include evaluating the performance of breeding lines, identifying stable source of resistance and transferring them to elite lines. The discovery and use of gall midge resistance genes should be made as efficient as possible because there are some biotypes for which effective resistance genes have not yet been found or for which resistance relies precariously on a single major gene (Katiyar *et al.,* 2004).

In Southern zone of Andhra Pradesh, incidence of rice gall midge was not reported by any researcher except in Nellore District. In Y.S.R District of the Southern zone rice gall midge incidence is increasing year by year and causing loss up to 25 to 60 per cent in yield and farmers face difficulty in managing this pest. So, gall midge biotype prevailing in Y.S.R District under field conditions and its molecular identification have to be carried out for development of resistance lines to that particular biotype.

Keeping in view of this above perspectives, the following research programme has been planned with specified objectives.

Objectives of investigation:

1. Survey for the incidence of rice gall midge across the Southern zone of Andhra Pradesh.
2. Detection of rice gall midge biotype under field conditions in Y.S.R District and its molecular identification.
3. Screening of rice genotypes against rice gall midge in Y.S.R District.

Chapter - II

Review of Literature

Chapter - II

REVIEW OF LITERATURE

Rice is the most important crop of the world and grown in 117 countries, and is a staple food of 3.5 billion people and more than 45% of the world population rely on rice as a staple food. Globally rice is planted in about 167.5 million ha and 504.5 metric tonnes of produce is harvested annually. Of this, Asia accounts for 90 per cent of the production and consumption of rice (FAO, 2020).

2.1 REDUCTION IN RICE YIELD DUE TO BIOTIC FACTORS

In India, annual yield losses were estimated to the tune of Rs. 6000-7000 crore, maximum loss being attributed to diseases (56%) followed by weeds (23%), insect pests (20%) and the rest by birds and nematodes (Raju, 2000). The rice plant is subjected to be attacked by more than 100 species of insects. Of these, 20 insects are considered as rice pests of economic importance that include stem borers, gall midge, defoliators and vectors such as leafhoppers and plant hoppers that cause both direct damage and also transmit various diseases (Pathak and Khan, 1994). The crop is exposed to pest attack from sowing till harvest. Insects damage plant parts by chewing plant tissues, boring into stems or sucking fluids from stem and grains. Damage caused by insect pests disturb physiology of plants and result in lowering the crop yield.

2.2 INFESTATION OF MAJOR INSECT PESTS AND YIELD LOSS IN RICE

Among the significant insect pests in Southeast Asia and China stem borer, brown plant hopper, gall midge and leaf hopper, whereas in South Asia gall midge, brown plant hopper and yellow stem borer are mostly responsible for significant losses in rice crop (Herdt and Riely, 1987).

According to Prasad *et. al*. (2007), yellow stem borer was responsible for an annual yield loss of 27–34%. According to Rai *et*. *al*. (2000), the prevalence of rice leaf folder caused yield losses of 90% in the fragrant cultivars Pusa basmati and Sugandha. According to Varma *et. al*. (2008), brown plant hopper might result in yield losses up to 20 to 60 per cent.

2.3 PEST STATUS AND DISTRIBUTION OF RICE GALL MIDGE

Rice gall midge *Orseolia oryzae* (Wood-Mason) is causing extensive damage in several rice growing countries of Asia *viz*., Thailand, China, Sri Lanka, India, Bangladesh, Pakistan, Burma, Kampuchea, Indonesia, Laos, Nepal and Vietnam. Earlier reports have documented prevalence of rice gall midge in African countries such as Sudan, Cameroon, Mali, Upper Volta, Ivory Coast, Senegal, New Guinea, Guinea - Bissau and Nigeria (Heinrichs and Pathak, 1981). It was first reported as an unidentified insect pest on rice in Monghyr District of Bihar State, India by Riley (1881). The pest was later identified as *Cecidomyia oryzae* Wood-Mason (Cotes 1889). Felt (1921) renamed the insect as *Pachydiplosis oryzae* and Gagne (1973) later named it as *Orseolia oryzae*.

After the introduction of the nitrogen-responsive high-yielding cultivars in the 1960s and their widespread adoption, the rice gall midge has extended its reach to various regions of Asia, such as Bangladesh, China, Cambodia, India, Indonesia, Lao PDR, Nepal, Sri Lanka, Thailand and Vietnam. Prior to 1982, it was believed that *O. oryzae* was the sole species that targeted rice however, Harris and Gange (1982) distinguished a distinct species of gall midge attacking rice in Africa, which they named *Orseolia oryzivora*. Details of the rice gall midge's widespread infestation across multiple countries, was provided in Table 2.1.

Rice gall midge, *O. oryzae* remained a key pest with wide spread occurrence up to 1990s and as a consequence of the emergence of six biotypes, this pest has caused serious losses in new areas like Bihar and North Eastern state of Manipur in addition to traditional areas of Odisha, Andhra Pradesh, Madhya Pradesh and Kerala (Fig. 2.1).

With respect to Andhra Pradesh, earlier it was reported in Ragolu and Nellore of Andhra Pradesh where biotype- 4 was prominent, in the recent years, its occurrence and wide spread damage is reported in Kurnool, Kadapa and Chittoor Districts of Rayalaseema, Chirala of Guntur, in and around areas of Maruteru.

Table 2.1. Infestation of rice gall midge in different countries

Country	Infestation levels / area	Reference
India	30-70%	Mathur and Rajamani (1984)
Thailand	60%	Hidaka *et. al.* (1996)
Indonesia	10%	Hidaka and Budiyanto (1984)
China	0.9 million ha	Di-yun and Cheng (1996)
Lao PDR	Severe infestation in 1993 and 1994	Khamhung (1996)
Sri Lanka	40%	Kudagamage *et. al*. (1988)
Vietnam	2000-5000 ha	Hung and Anh (1996)
Africa	45-80%	Ukwungu *et. al*. (1989)
Cambodia	Up to 59%	Jahn and Bunnarith (2004)

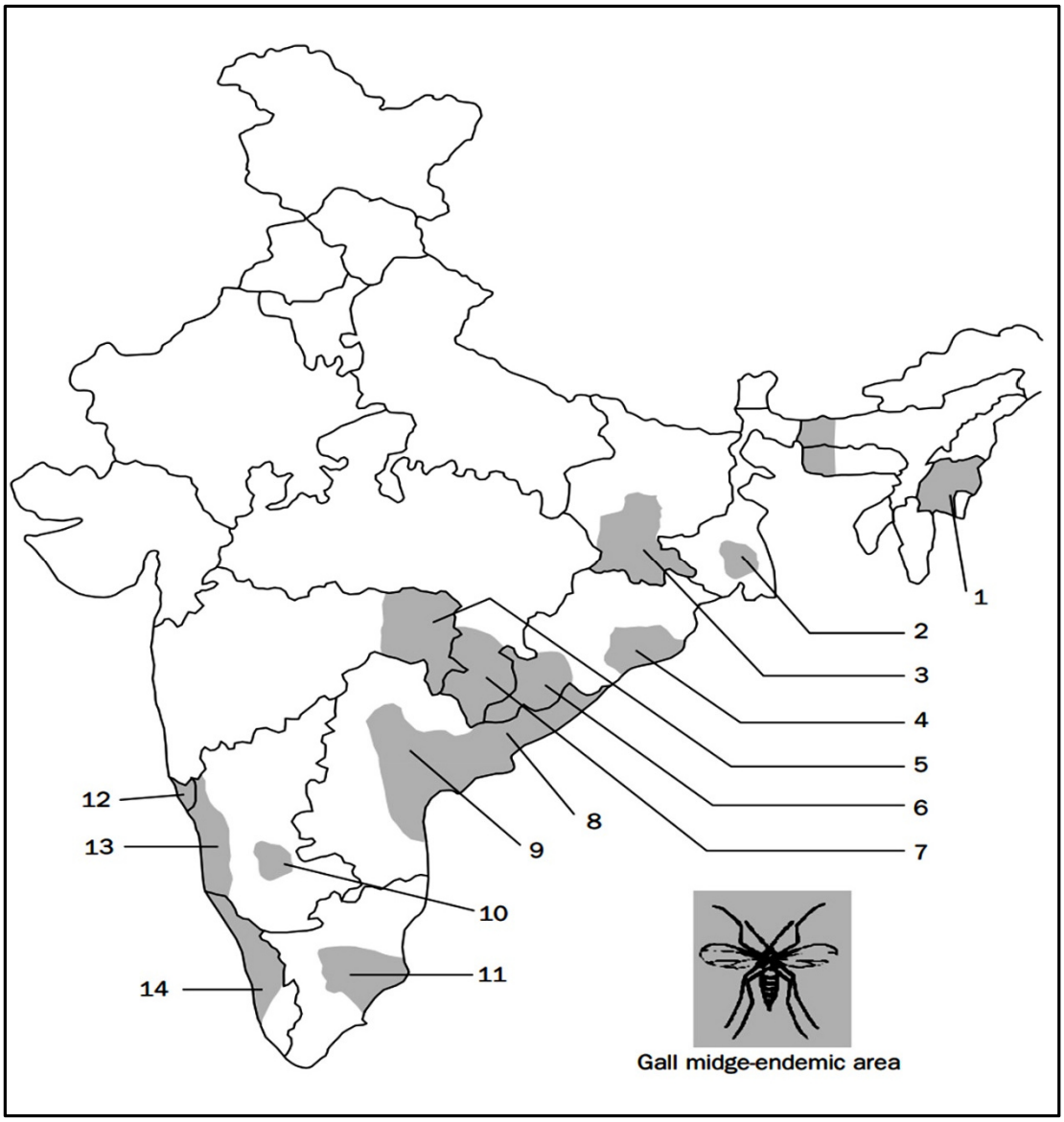
(Source: Mathur and Krishnaiah, 2004)

2.3.1 Ecology of Rice Gall Midge

Gall midge infestation occurs when the early rains comes, makes the adult flies active. During, subsequent dry periods and under delayed planting conditions, the pest population multiplies on grasses and the flies migrate in large numbers to the late planted rice crop. Cloudy skies and drizzling rains are conducive to the fast buildup of gall midge populations. The favorable condition for fly development is 26-30°C and 82-88 per cent relative humidity. Heavy rain storms cause high mortality. The insects are less abundant in crop years preceded by a warm and dry spring.

The maggots can live under submergence for several days, changes in water levels in the rice fields do not have a distinct effect on fly incidence (Harathi, 2019).

Jagadeesha *et. al.* (2009) reported that during the off season the pest survived on sprouts from the left-over rice stubbles after the harvest of the paddy crop. None of the plants growing wild around the plots served as alternate hosts for the pests at both Mangalore and Mandya.



Source: Mathur and Krishnaiah, 2004

1	Manipur	8.	Andhra Pradesh- North coastal
2	West Bengal	9.	Warangal - Karimnagar
3	Jharkhand- Ranchi region	10.	Karnataka- Madhya
4	Odisha- coastal	11.	Tamil Nadu- Madurai
5	Maharashtra- Bandara region	12.	Goa
6	Odisha- Sambalpur	13.	Karnataka- coastal
7	Chhattisgarh- Raipur	14.	Kerala- Kuttanad

Fig. 2.1 Map showing endemic area for rice gall midge in India

2.3.2 Alternate Hosts for Gall Midge

In the off season the pest thrives on the alternate hosts such as *Leersia hexandra* (Natarajan *et al.,* 1989), *Echinochloa crusgalli* (Sain and Kalode, 1988) and wild races (Israel *et al.,* 1963) *Oryza nivara* (Hidaka *et al.,* 1983), *O. barthii* (Srivastava, 1986), *O. rufipogon*, *O. perennis* and *O. glaberrima.*

Several alternate host plants recorded in India and Thailand included wild rice *O. officinalis* and several graminaceous weed plants such as *Ischaemum aristatum, E. colona, Paspalum* spp. and *Leersia* spp. (Pathak and Khan, 1994).

Mardi *et. al.* (2009) observed the presence of rice gall midge in three grasses *Eleusine indica, Bothriochloa* spp. and *Paspalum* spp. which acted as alternative host to rice gall midge. Highest infestation was observed on *E. indica* during the second week of July.

2.3.3 Physiology of Gall Formation and Damage due to Rice Gall Midge

The damage caused by the rice gall midge is due to the formation of 'silver shoot,' a silvery white, tubular leaf sheath (gall). These galls develop from regular tillers and result in poor crop yield as they dry out without bearing panicles. Gall formation occurs when leaf primordial differentiation is suppressed at the growth cone, leading to the development of radial ridges and elongation of the leaf sheath. Early infestations lead to excessive tillering, but the new tillers are often infested and only few produce panicles. The insect's salivary gland secretes an unidentified chemical called 'cecidogen,' which is believed to contribute for gall formation. Plant growth regulators such as indole acetic acid (IAA) is involved in gall formation, as suggested by tissue proliferation following IAA application and midge infestation. Higher levels of IAA and tryptophan were observed in gall tissue compared to healthy plants (Balasubramanian and Purushothaman, 1971). The insect's saliva contains IAA, acting as a gall inducer and polyphenol oxidase (PPO) in the saliva plays a role in gall development by interacting with phenolic compounds and is fundamental in gall development (Ananthakrishnan, 1998).

The rice gall midge infestation starts in the seedbed and continues until the booting stage. Maggot of the pest mature only on growing primordia and cannot

survive beyond the vegetative stage. Galls appear within 3-7 days after maggot enter the plant's growing point, measuring 1-2 cm wide and 10-30 cm long, occasionally reaching 50 cm.

The maggot feed at the base of the growth cone and only one survives if multiple maggots reach a shoot apex. Gall formation is a result of growth cone suppression and radial ridge development from the innermost leaf primordium, followed by leaf sheath elongation. Nutrient diversion and larval substances stimulate radial ridge and gall growth. First-instar maggot develops in active shoot apices, while inactive axillary shoot apices are inhibited. High population pressure leads to infestation of all shoot apices, causing staggered gall formation and adult emergence due to larval dormancy. Dormant first-instar maggot at inactive axillary shoot apices contribute to population carry-over between seasons. During the dry season, dormant first-instar maggot can develop normally in the following wet season. Multiple infestations of shoot apices result in staggered maggot development and adult emergence, influenced by weather conditions (Nalini and Henry, 1968).

The interaction between gall midge and rice can be compatible or incompatible. In a compatible interaction, virulent maggots feed on a susceptible host's meristematic region, forming leaf-sheath galls that inhibit panicle formation. In an incompatible interaction on a resistant host, avirulent insects cannot sustain feeding and die, allowing normal plant development (Bentur and Kalode, 1996).

2.3.4 Biology of Rice Gall Midge

Studies by Sain and Kalode (1988) suggest that the insect produces unisexual progenies, *i.e.,* a female produce either all-female or all-male progeny. The rice gall midge exhibits karyological sexual dimorphism, with six chromosomes in the metaphase ganglionic cells of larval populations generating males and eight chromosomes generating females. Recent research suggests that sex determination depends on the presence of a bacterium, *Wolbachia* spp. (Sahu *et al.,* 1996). Continuous rearing of the insect leads to inbreeding, resulting in the emergence of only females and subsequent population decline. Female rice gall midges emit a strong sex pheromone (Sain and Kalode, 1985).

According to Archana (2011), the incubation period of *O. oryzae* ranged from 1.5 to 3.5 days, with an average of 2.50 ± 1.41. Favourable microclimatic conditions led to faster growth of maggots, which completed development within 8.5 to 9.5 days, with a mean of 9.00 ± 0.71. The pupal period inside the gall ranged from 3.5 to 4.5 days, with an average of 4.00 ± 0.71. Male adult longevity lasted between 1.0 to 1.5 days, with an average of 1.25 ± 0.35, while females lived longer, ranging from 1.5 to 2.5 days, with an average of 2.0 ± 0.71. Females laid an average of 96.5 ± 14.85 eggs within a day or two, ranging from 86 to 107 eggs. The entire life cycle of *O. oryzae* lasted between 15 to 20 days, with an average of 17.50 ± 3.54.

2.3.5 Yield Loss due to Rice Gall Midge

Israel *et. al.* (1959) developed a regression technique to calculate the damages caused by rice gall midge infestation. According to their findings, every one per cent increase in infestation results in a yield loss of 0.50 per cent, which amounts to 23.71 pounds per acre. Various researchers estimated the lists of anticipated yield loss by rice gall midge (Table 2.2).

On an average, rice gall midge causes loss in yield in susceptible cultivars to extent of 3 to 70 per cent, at times causing 100 per cent loss of the crop when pest population is very severe under favourable condition (Chatterji *et al.,* 1976).

In India, gall midge is considered the third most significant pest of rice, capable of causing crop losses ranging from 10 to 100 per cent. (Siddiq, 1991). Based on estimates available for Eastern India (Widawsky and O'Toole, 1996) and Southern India (Ramasamy *et al.,* 1996), gall midge causes an annual yield loss of about 477,000 tonnes of grains or 0.8% of the total production, amounting to US $ 80 million.

According to a report by Lima *et. al.* (2007), gall midge infestation in India has resulted in an annual crop loss of approximately 28-35 per cent.

Table 2.2. Yield loss due to infestation of Rice Gall Midge

Item	Reference
For every 1% increase in SS, 0.5% yield loss (traditional variety GEB 24)	Israel *et. al.* (1959)
For every tiller infected, an additional tiller produced but with low productivity (Var. GEB 24)	Israel and Prakasa Rao (1968)
For every 1% increase in SS, 0.4% reduction	Prem Chand and Acharya (1983)
Same level of infestation (25% SS) 7.9 to 32.9% yield in different varieties	Katanyukul *et. al.* (1980)
For each 1% damage, 0.92% grain yield loss	Hidaka *et. al.* (1974)
For each 1% increase in SS, yield loss of 1.03 q/ha	Sundararaju (1986)
For every 1% increase in SS, 0.06% in 1014 to 1.1% in CR 58-MR-1530	Prakasa Rao and Kittur (1989)
For each 1% increase in SS, yield loss of 0.7% to 0.72%	Venugopal Rao *et. al.* (1990)
For every 1% increase in SS, yield loss of 0.29% to 1.07	Kudagamage *et. al.* (1988)

Source: Adopted from Rice gall midge: pest status, distribution and yield losses by Mathur and Krishnaiah (2004).

2.4 SURVEY FOR THE INCIDENCE OF MAJOR PESTS OF RICE IN INDIA

Jharkhand state is known to be endemic to gall midge over past several decades. However, pest incidence is mainly influenced by the weather factors. During 2011, the entire state experience severe incidence of gall midge. Field experiments were laid out in farmers field in pest endemic regions of the state to evaluate performance of local popular rice varieties and available hybrids. Results revealed relatively low pest damage in popular varieties like Lalat, IR36, Naveen, Sahbhagi Dhan, Sudha and Abhisek. But all the hybrids tested were found to be highly susceptible to the pest (Prasad, 2011).

In western Uttar Pradesh, a survey was conducted on insect-pest incidence in the Basmati rice ecosystem. The predominant varieties grown are Pusa Basmati-1121, Pusa Basmati-1, Pusa Basmati-6, Pusa Basmati-1509 and Vallabh-22 Basmati. The crop is infested by various insect-pests, including leaf folder and stem borer, which significantly impact rice production. Natural defenders like dragonflies, spiders, and praying mantises were also observed, along with other insect-pests such as gundhi bug, rice hispa, brown plant hopper and grasshoppers (Gangwar *et al*., 2015).

A research study was carried out in Phulwarisharif Block of Patna District to investigate the prevalence of major insect pests in rice cultivation. The main rice variety grown in the region was BPT-5204, which constituted 70 per cent of the cultivated area. The study involved monitoring major insect pests every two weeks to analyse their population dynamics and the extent of damage caused in different plots. The fortnightly observations were intended to obtain information on the correlation between pest population and meteorological changes. The study revealed that major insect pests significantly reduced the production of BPT-5204 rice in the area, highlighting their crucial role in the crop's decline (Surendra and Bindu, 2016).

A study conducted from February 2012 to November 2014 in Burdwan District, West Bengal, recorded 32 arthropod species under eight orders from different rice fields. The populations of various insect pests varied between the locations of Galsi and Memari, across dry and wet seasons. For example, the population of *S. incertulas* (yellow stem borer) ranged from 6.33 to 7.33 larvae per hill in the dry season and from 2.67 to 3.67 larvae per hill in the wet season during the years 2012 to 2014 (Ghosh *et al*., 2016).

During the *kharif* season of August to December 2013, a fixed plot survey was conducted to assess insect pests and natural enemies in the paddy ecosystem across various locations in the Cauvery command areas of Mandya District, near the Vishveshvaraiah canal farm. Among the pests observed, yellow stem borer, plant hoppers, gall midge, and leaf folder were particularly severe. On the other hand, mirids and spiders were identified as the most important natural enemies among the predators. The average estimated yield loss in rice due to these pest

infestations ranged from 21 to 51 per cent. These findings highlight the significance of managing insect pests and fostering the presence of beneficial natural enemies to minimize yield losses and promote sustainable rice production in the region (Parasappa *et al*., 2017).

In the Balaghat District, a study was conducted on the incidence of major insect pests in fine rice (short cylinder) variety, popular from a private company. The study covered a cultivated rice-growing area of 60-70 thousand ha. The observations were carried out during the *kharif* season of 2017-2018. Fortnightly monitoring of pest populations was done in conjunction with changes in meteorological data. The survey covered various stages of paddy reproduction, including vegetative milking, to assess the intensity of damage caused by insect pests during each stage. The findings revealed that the crop was infested by major insect pests, resulting in a decrease in rice production yield (Bisen *et al*., 2019).

A rowing survey was conducted in Karnataka's Cauvery and Kabini command area examined gall midge incidence in paddy cultivation. Jaya, Jyothi, MTU 1001, BR 2655, Tanu and Gangavathi sona were the predominant rice varieties Jaya was covering around 50 per cent of the total area. The study found significant gall midge infestation affecting rice production. Mandya had the highest infestation at 9.03 per cent silver shoot damage, followed by Chamarajanagar (8.7%), while Mysore had the lowest at 6.95 per cent. Infestation leads to sterile tillers and yield loss, with stunted growth in the nursery. Cultivating resistant varieties is the recommended cost-effective approach to control gall midge (Mamathad *et al*., 2020).

In the past, major insect pests were identified based on the damage they caused, affecting 10 per cent of paddy plants in various aspects. Continuous surveys were necessary to justify the pest status in paddy fields after several years. The roving survey was conducted during the *kharif* season of 2014-15 in different Blocks of Patna District. The research work involved field surveys of paddy in different Blocks to find out the major insect pests. Among the surveyed Blocks, Bakhtiyarpur Block and Phulwarisharif Block in Patna District were the most affected. Green Leaf Hopper played a major role in infestation, accounting for

34.61 and 32.20 per cent in Bakhtiyarpur and Phulwarisharif Blocks, respectively (Surendra and Bindu, 2020).

2.5 DETECTION OF RICE GALL MIDGE BIOTYPE UNDER FIELD CONDITIONS

Anbuselvi (2003) conducted a study on the virulence characteristics of the Raipur gall midge population using differential rice varieties TN1, Phalguna (Gm2) and Kavya (Gm1). The study showed that out of 165 female insects, 12 insects (7.22%) produced virulent offspring on Phalguna (Gm2), while only one virulent offspring was produced on Kavya.

Initially, Asian gall midge biotypes were identified based on the reaction to a set of differential rice varieties categorized into three groups. Further study of the genetic basis of resistance in these differentials led to the addition of another group of differentials, including a susceptible check with no resistance gene. This revised set helped to identify three more biotypes. In total, six distinct gall midge biotypes have been characterized in different parts of India, based on the field reaction to the set of differentials. A new seventh biotype was recently characterized from the population at Warangal in the state of Andhra Pradesh. (Vijaya Lakshmi *et al.,* 2006).

Identification and virulence composition of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) biotypes in Kodagu, Mysore and Hassan districts, South Karnataka, South India was studied during 2005 - 2006 under field conditions. Rice gall midge infestation in these three districts was detected for the first time in 2004 wet season. A set of 14 standard rice differentials representing four groups identified to characterize the prevailing rice gall midge biotypes in the country were evaluated against local gall midge populations in four locations. Based on the reaction pattern of standard differentials to gall midge populations, the biotype 1 gall midge was detected with R-R-R-S reaction pattern. The virulence composition of Asian rice gall midge, *Orseolia oryzae* was studied by using three standard differentials *viz.,* W1263 (*Gm1* gene for resistance), Phalguna (*Gm2* gene for resistance) and TN1 (susceptible without any gene). Based on the reaction pattern of standard differentials to gall midge populations, the biotype 1 population was

detected at Madikeri and Ponnampet, the populations expressed their virulence only against susceptible group consisting TN1 differential. The female to male sex ratio of their F_1 was close to 3:1 in TN 1, these results clearly indicated the prevalence of genetically homogeneous biotype-1 population with avirulent to both *Gm1* (W1263) and *Gm2* (Phalguna) genes for resistance (Lingaraj *et al.,* 2008).

According to the annual report of IIRR in 2017, the biotype present at their location in Hyderabad was characterized by the pattern of resistance-susceptibility-resistance-resistance-susceptibility (R-R-R-R-S). The populations at Jagdalpur also followed this same pattern, while the DUOKANG-1 and BG308-2 populations showed susceptibility at IIRR. Meanwhile, the populations at Jagtial and Ranchi exhibited the typical pattern of resistance-susceptibility-resistance-resistance-susceptibility (R-S-R-R-S) for biotype 3, except for the susceptibility of RP 2068-18-3-5 at both locations.

Seven distinct biotypes of the Asian rice gall midge have been characterized so far from different parts of India. Warangal rice gall midge population is designated as biotype 4M. In order to find the virulence pattern of the rice gall midge population, single gall midge female virulence test was conducted at RARS, Warangal with three differentials, W1263 (Gm1), RP2068-18-3-5 (gm3), Aganni (Gm8) along with Purple (Susceptible check) and gene pyramided line (Gm4, Gm8 and gm3) of F3 generation of inter-cross, (MTU 1010 × RMSGM3) × (MTU 1010 × RP 5923). 56% of the females were virulent among which 53.57% were virulent on purple, 50% on gene pyramided line (Gm4, Gm8 and gm3), 32.14% on W1263 (Gm1), 30.35% on RP2068-18-3-5 (gm3) and 7.14% on Aganni (Gm8). Sex ratio of off springs emerged is 1:3 and is favourable in all the differentials and gene pyramided line except W1263. It was found that Aganni (Gm8) has recorded low virulence by gall midge biotype 4M (Sahithi *et al*., 2018)

In 2016-18, the identification of the Asian rice gall midge biotype was conducted under field conditions at ARS, Nellore, using a set of 17 standard rice differentials representing five groups identified to characterize prevailing biotypes in the country. The reaction pattern of the gall midge population to the differentials

revealed that the biotype at ARS, Nellore did not follow any of the seven identified biotypes. To determine its similarity to other biotypes, the per cent similarity index was calculated. Based on two consecutive years data, it was found that the biotype at ARS, Nellore had a 70.8 per cent similarity to biotype 6 (Harathi, 2019).

In India, seven distinct biotypes of the Asian rice gall midge *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae) population have been characterized so far from different parts of India and the gall midge population under natural field conditions at RARS at Warangal was designated as biotype 4M. A set of 16 standard rice differentials representing five groups already identified to characterize the prevailing rice gall midge biotypes in the country were evaluated against gall midge population. Based on earlier studies, resistance against biotype 4M confirmed only through the group IV differentials with resistance genes *viz.,* gm3, Gm4 and Gm8. The results of present study revealed that the reaction of differentials of group I, II, III and V (TN1, Susceptible check) showed susceptibility during both the years *i.e*., *kharif*, 2011-12 and *kharif*, 2012-13 whereas, reaction of group IV differentials was not stable and changed year after year. Based on study, they indicating a change in the reaction pattern of standard differentials to gall midge biotype 4M with virulence against the resistant group IV rice differentials (Sunitha *et al*., 2021).

Virulence composition of traditionally designated biotype 2 field population of Asian rice gall midge was conducted a decade after in 2019 and 2020 at coastal Karnataka, India using three standard differentials *viz.,* W1263 *(Gm1* gene for resistance), Phalguna *(Gm2* gene for resistance) and TN1 (susceptible without any gene). The local population of gall midge was virulent against all 16 standard rice gene differentials representing four groups identified to characterize the prevailing rice gall midge biotypes in India. In south coast, 73.33 to 87.27 per cent population showed virulent attributes of traditional biotype 2 designated in 1989. Whereas in north coast, 79.69 to 86.36 per cent population exhibited virulence attributes towards new biotype 3 for the first time in the state of Karnataka, India. These results suggested a progressive change in the traditionally designated population of biotype 2 capable of damaging resistant varieties in the region for over three decades. Further, the single female test for

their F1 progenies in all endemic locations indicated an evolution of new biotype of rice gall midge in the region (Vijaykumar *et al.,* 2022).

Field experiments to identify the prevailing rice gall midge biotype were conducted at the Agricultural Research Station, Nellore during *kharif*, 2015-16 to 2021-22 by using 17 standard differentials from IIRR, Hyderabad. During 2015 (AGANNI and INRC 15888), 2019 (AGANNI and INRC 3021) showed resistance and 2021 (AGANNI, INRC 15888 and INRC 3021) showed susceptible with this changing reaction and followed the high similarity index for biotype VI (Paramasiva *et al.,* 2023).

2.6 MOLECULAR IDENTIFICATION OF RICE GALL MIDGE

Thirteen years of evaluation were conducted on fourteen differential rice cultivars, grouped into four major donor groups, at 11 field locations across seven Indian states. The evaluation was focused on their resistance to the rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae). Based on their reaction patterns against the Eswarakora and Siam 29 differential groups, three distinct biotypes (1, 2, and 3) were identified. Biotype 1 does not damage entries involving either Eswarakora or Siam 29. Biotype 2 can damage the Eswarakora group and its derivatives but not the Siam 29 group. Biotype 3, on the other hand, can damage the Siam 29 group while being unable to do so with the Eswarakora group (Kalode and Bentur, 1989).

Ehtesham *et. al*. (1995) isolated a randomly repeated genomic DNA clone from the rice gall midge. Hybridisation of this repeat 14 to the restriction enzyme digested genomic DNA blots of biotype 1, 2, 3, 4 and 5 revealed polymorphisms. This repeat element can be used to differentiate different biotypes at molecular level.

Behura *et. al.* (1999) developed a polymerase chain reaction (PCR) based assay that distinguished five different biotypes of the Asian gall midge (*Orseolia oryzae*), a major insect pest of rice. A total of 400 random primers were screened using random amplified polymorphic DNAs (RAPDs). Five diagnostic PCR products were isolated, cloned, sequenced and converted to sequence characterized amplified regions (SCARs). Primers specific to these SCARs were

able to amplify specific DNA fragments from genomic DNAs of five biotypes of gall midge in a multiplexed-PCR-based assay. The amplified DNA fragments were used as diagnostic markers to identify different biotypes of gall midge. The SCAR primers were also capable of differentiating the Asian from the African rice gall midge (*Orseolia oryzivora*) as well as detecting a variant of biotype 5 which caused an outbreak in Kerala, India.

Anbuselvi (2003) conducted a study on the differentiation of biotypes in the Chhattisgarh and North eastern regions using SCAR markers. The study found that although all biotypes shared a 0.55 bp fragment, the SCAR markers successfully distinguished between biotypes 1 and 6 by exhibiting biotype-specific bands for each.

Vijaya Lakshmi *et. al.* (2006), reported the population of gall midges from Warangal has developed resistance against it. A total of 400 random primers were screened using random amplified polymorphic DNAs (RAPDs). Five diagnostic PCR products were isolated, cloned, sequenced and converted to sequence characterized amplified regions (SCARs). Primers specific to these SCARs were able to amplify specific DNA fragments from genomic DNAs of five biotypes of gall midge in a multiplexed-PCR-based assay. The amplified DNA fragments were used as diagnostic markers to identify different biotypes of gall midge. The SCAR primers were also capable of differentiating the Asian from the African rice gall midge (*Orseolia oryzivora*) as well as detecting a variant of biotype 5 which caused an outbreak in Kerala, India, a rice variety that previously showed resistance to gall midges due to its Ptb21 source cultivar. This resistance pattern was not observed in the six biotypes previously characterized and as a result, this new biotype is currently referred to as biotype 4M.

After the widespread cultivation of high yielding gall midge-resistant rice varieties in farmer fields, different populations or biotypes were observed (Singh, 1996). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is a cause for concern. So far, seven biotypes (GMB1 to GMB6 and GMB4M) of gall midge and 11 gall midge resistance genes (Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9,

Gm10 and Gm11) have been identified (Vijaya Lakshmi *et al.,* 2006 and Himabindu *et al*., 2010).

Bentur *et. al.* (2011) conducted inheritance studies using three SSR markers and found that two markers, Oosat55 and Oosat59, exhibited sex-linked inheritance, while one marker, Oosat43, showed autosomal inheritance. The researchers suggest that these markers were valuable for developing integrated pest management strategies and studying the evolution of biotypes in this significant rice pest.

The complete mitochondrial genome of the Asian rice gall midge, *Orseolia oryzae* (Diptera: Cecidomyiidae) was sequenced, annotated and analysed in the present study. The circular genome is 15,286 bp with 13 protein-coding genes, 22 tRNAs and 2 ribosomal RNA genes and a 578 bp non-coding control region. All protein coding genes used conventional start codons and terminated with a complete stop codon. They evaluated the number of iterations of the tandem repeat elements found in the mitogenome. That led to the identification of genetic markers capable of differentiating rice gall midge biotypes (Atray *et al.,* 2015).

DNA barcode was developed, and the phylogenetic status of the Asian rice gall midge (*O. oryzae*) within the Cecidomyiidae family was determined. *Feltiella acarivora* was found to be the nearest relative to *O. oryzae*, while *Resseliella yagoi* was identified as the distant relative. The mitochondrial DNA of *O. oryzae* exhibited a clear bias toward AT composition, showing similarities in the insect mtDNA's observed AT composition. Furthermore, this study revealed variations in the nucleotides within each codon position of *O. oryzae*, particularly in the strong and less constrained positions of codons when compared with other species in the Cecidomyiidae family. These findings provide valuable insights into the genetic characteristics and relationships of the Asian rice gall midge and its relatives, aiding in better understanding and management of this damaging pest (Kattali *et al.,* 2015).

2.7 SCREENING OF RICE GENOTYPES AGAINST RICE GALL MIDGE

Ukwungu *et. al.* (1999), stated incorporating resistant rice varieties seems to be the most efficient component for integration into a pest management plan.

In order to know the reaction of popular high yielding varieties (HYVs) and few improved genotypes to rice gall midge, 87 genotypes were screened under natural field condition at 50-62 days after transplanting at zero and 120 kg N ha^{-1} based on appearance of silver shoot. Few genotypes from early group like Ananga, Annada, Kharavela and Shaktiman showed highly resistant reaction at both the level of nitrogen with zero per cent silver shoot. Cultivar Jajati and Suraksha showed moderately resistant reaction in mid group and Chaitanya in late group. Other cultivars/ genotypes showed high infestation of gall midge as observed from the incidence of silver shoots and are classified as either susceptible or highly susceptible at 120 kg N ha^{-1} level. Under high N level invariably there was high incidence of gall midge in most of the genotypes than the zero N level (Meher *et al.,* 2009).

Dutta *et. al.* (2014) studied the genetic diversity of resistance in 100 rice genotypes under field condition against gall midge biotype GMB4M at Warangal and GMB1 in greenhouse at DRR. A set of 77 gall midge resistant genotypes was collected from ARS, Ragolu, Andhra Pradesh (A.P), India. These donor lines have been showing resistance with nil damage through successive years of testing and seed multiplication under natural pest infestation at pest endemic location.

Atanu Seni and Bhima Sen Naik (2017) reported that certain rice genotypes, including W 1263, INRC 3021, Sudu Hondarawala, PTB 26, RP4686-48-1-937, RMSG-11, WGL 1147, WGL 1127, WGL 1121, WGL 1131, WGL 1141 and JGL 27058, exhibited resistance against rice gall midge.

Mangala Parikh *et. al.* (2017) reported that Dubraj (D:1251), Mancha (M:1028), Mahipal (M:27 A) and Krishnanjana showed resistance to stem borer, and Shrikamal (S: 660 I) demonstrated resistance to gall midge in tests conducted at Ambikapur and Jagdalpur. These promising genotypes can be developed into

varieties or utilized in breeding programs to address these pests, which pose significant threats to rice crop.

Shravan Kumar *et. al*. (2020) screened 173 rice entries against gall midge [*Orseolia oryzae* (Wood-Mason)] for resistance at Regional Agricultural Research Station, Warangal during *kharif* 2019 under delayed planting situation ensuring sufficient pest load. Among 173 rice entries screened, three entries *viz.,* IBT MRR 18, IBT MRR 23 and IBT MRR 24 were found highly resistant and six entries *viz.,* IBT MRR 17, IBT MRR 19, IBT MRR 20, IBT MRR 21, IBT MRR 22 and IBT MRR 28 had shown resistant reaction against gall midge. These entries can be used in breeding programmes as a source of gall midge resistance.

Malathi and Shravan Kumar (2021) in the wet season in 2016, 2017, and 2018, a total of 18, 23 and 21 entries respectively were screened for resistance against Asian rice gall midge at Professor Jayashankar Telangana State Agricultural University, Regional Agricultural Research Station, Warangal, Telangana. The screening was conducted under delayed planting situation to ensure sufficient pest load. Only one entry, WGL-811, showed a resistant reaction with 5 per cent hill damage and 0.34 per cent silver shoots. Five entries, including RDR 1162, WGL 1119, IBT R4, WGL 1150 and WGL 1021, showed moderate resistance with less than 5 per cent silver shoot damage. Among these, WGL 811, WGL 1119 and WGL 1150 recorded lower plant damage (5-25%) and silver shoot damage, making them promising candidates for breeding programmes as a source of gall midge resistance or potential varieties, depending on their yield traits and plant type.

A total of 52 rice genotypes along with resistant and susceptible checks were evaluated against rice gall midge biotype 1 under greenhouse conditions at the ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad and under field conditions against biotype 3 at the Regional Agricultural Research Station (RARS), Jagtial and biotype 4M at RARS, Warangal during wet seasons 2014 and 2015. Five germplasm lines *viz.,* CR 2613-1-1-1-5-1, CR 2615-1, JGL 11470, JGL 11727 and Sukaradidhan-1 were identified as resistant to biotype-1 (Anusha *et al.,* 2022).

In *kharif* 2021, a study was conducted at the Regional Agricultural Research Station (RARS), Jagtial to evaluate 84 rice germplasm lines against biotype 3 of the rice gall midge (*Orseolia oryzae*) along with resistant and susceptible checks. Six entries, namely KAKAI, SINNA SIVAPPU, PTB-12, WGL-1145, WGL-1147 and WGL-1127 showed high resistance with a score of 0 against gall midge. Four entries, namely IR72476-B-P-9-3-1-1, RP 5332-54-11-8-2-13, WGL-1143 and SUDD HONDARAWALA, showed resistance with a score of 1 against gall midge (Kumar *et al.,* 2022).

During *kharif* 2016, nineteen rice varieties, released from the Regional Agricultural Research Station (RARS), Warangal, Telangana, India, were screened for gall midge resistance under both phenotypic and genotypic conditions. Additionally, one susceptible check (TN1) was also included in the screening process. Among the 19 rice varieties tested, Sheetal exhibited a highly resistant reaction to gall midge at both the field level and the genotypic level, possessing three gall midge genes, namely gm3 (Gm3del3), Gm4 (Gm4 LRR) and Gm8 (PRP). Varieties such as Orugallu, Bhadrakali, Shiva, Kesava and Ramappa showed a moderate level of resistance to gall midge in the field and possessed only the gm3 gene, while WGL-915 exhibited a moderate level of resistance to gall midge in the field and possessed only the Gm4 gene (Hari *et al.,* 2022).

83 elite rice genotypes were tested for resistance to rice gall midge [*Orseolia oryzae* (Wood- Mason)] in the field at Regional Agricultural Research Station, Warangal, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Telangana during *kharif*, 2021. Among 83 rice genotypes screened, WGL-1789, WGL-1790, WGL-1798 and WGL-1800 were found highly resistant and WGL-1767, WGL-1778, WGL-1782 and WGL- 1792 were found to be resistant to gall midge. These promising resistant entries could be utilized as donors in breeding programmes aimed at development of gall midge resistant varieties (Kumar *et al*., 2022).

Total 115 rice genotypes were screened against gall midge incidence at 30 and 50 days after transplanting by standard evaluation system (scale for scoring). Highest incidence of silver shoot was recorded in TN-1 (55.34%) at 50 DAT.

Whereas, 28 entries *viz*. JGL 35161, JGL 36175, JGL 38168, KNM 12368, KNM 12424, Aganni, KNM 12450, SKL 11-3-838-220-10-150, WGL 1614, WGL 1623, FBL 19064, FBL 19101, FBL 19102, FBL 19112, Karma Mashuri, Mahamaya, GM 4 (IBT), GM 40 (IBT), WGL1 (IBT), WGL2 (IBT), WGL3 (IBT), WGL 31 (IBT), RP 5923 (IBT), APKS 83–24, APKS 83–40, ENT GP 2018-178 and RP 6290-20-36 showed 'Nil' damage and found highly resistance to gall midge (Darro *et al.,* 2023).

Chapter - III

Material and Methods

Chapter – III

MATERIALS AND METHODS

The present investigations on the **"Survey, varietal screening and molecular identification of Asian Rice Gall Midge in Y.S.R District of Andhra Pradesh"** was carried out at Agricultural Research Station, Utukur, Kadapa (latitude 14.4538°N and longitude 78.8123°E). Procedures followed and materials used in these studies are presented here under.

3.1 SURVEY FOR THE INCIDENCE OF RICE GALL MIDGE ACROSS THE SOUTHERN ZONE OF ANDHRA PRADESH.

Roving survey were conducted in major rice grown areas of Southern zone of Andhra Pradesh during *kharif* in Y.S.R District and *rabi* in Chittoor and Nellore Districts, 2022 to know the severity of incidence of Rice Gall Midge (RGM) and collected the silver shoots having the immature stages from the surveyed regions for further molecular studies.

3.1.1 Area surveyed

Three major rice growing districts of Southern zone Andhra Pradesh *viz*., Chittoor, Y.S.R and Nellore districts were selected for survey on incidence of RGM. In each district, three mandals were selected, in each mandal two villages were selected and in each village, five farmer's fields were selected. The list of districts, mandals and villages surveyed are mentioned in Table 3.1 (Plate 3.1) (Fig. 3.1).

3.1.2 Survey method

Survey was conducted for the incidence of RGM on rice crop in farmers field in different districts of Andhra Pradesh during, 2022. Field scouting was done in each location, the infestation of RGM was recorded at vegetative stage, across varieties cultivated in respective farmer's field. In each location, the survey plot area was divided into four quadrants of approximately 200 m^2. In each quadrant

20 hills were selected diagonally for observation, the information regarding the varieties were confirmed by the local farmers (Appendix-I).

3.1.3 Assessment of damage caused by RGM

The observations on the gall midge infestation were recorded by counting the total number of plants with silver shoot and total number of tillers on hill basis, fields and expressed as per cent silver shoot and plant damage. Scoring of *Orseolia oryzae* plant damage was done as per the Mamathad *et. al*. (2020) and these parameters were calculated by using the following formulas.

$$\% \text{ Damaged plants} = \frac{\text{Total number of infested plants}}{\text{Total number of plants}} \times 100$$

$$\% \text{ Silver shoots} = \frac{\text{Total number of tillers with silver shoots}}{\text{Total number of tillers}} \times 100$$

3.1.4 Collection of insect samples

Insect immature stages of *Orseolia oryzae* populations were collected from each district during the survey period to study the molecular identification of collected insects. During the survey, rice gall with either maggots or pupal stages were collected into the sterile centrifuge tubes (50ml) without causing damage to the insect and the galls were brought to the laboratory within 3-4 hours of collection and transferred to the test tubes immediately and kept for adult emergence from galls. Adults were collected during the next day, kept in a tube having 70% alcohol at -20^{o}C till further use for molecular identification (Plate 3.2 and 3.3).

3.1.5 Analytical tools and techniques employed

Simple statistical tools like mean and percentage were used to analyse the data using Microsoft Excel 2021 Spread Sheet.

Chittoor District

Y.S.R. District

Nellore District

Plate 3.1: Survey for the incidence of rice gall midge, *Orseolia oryzae* in Southern zone of Andhra Pradesh

Plate 3.2: Collected galls with immature stages of rice gall midge during the survey

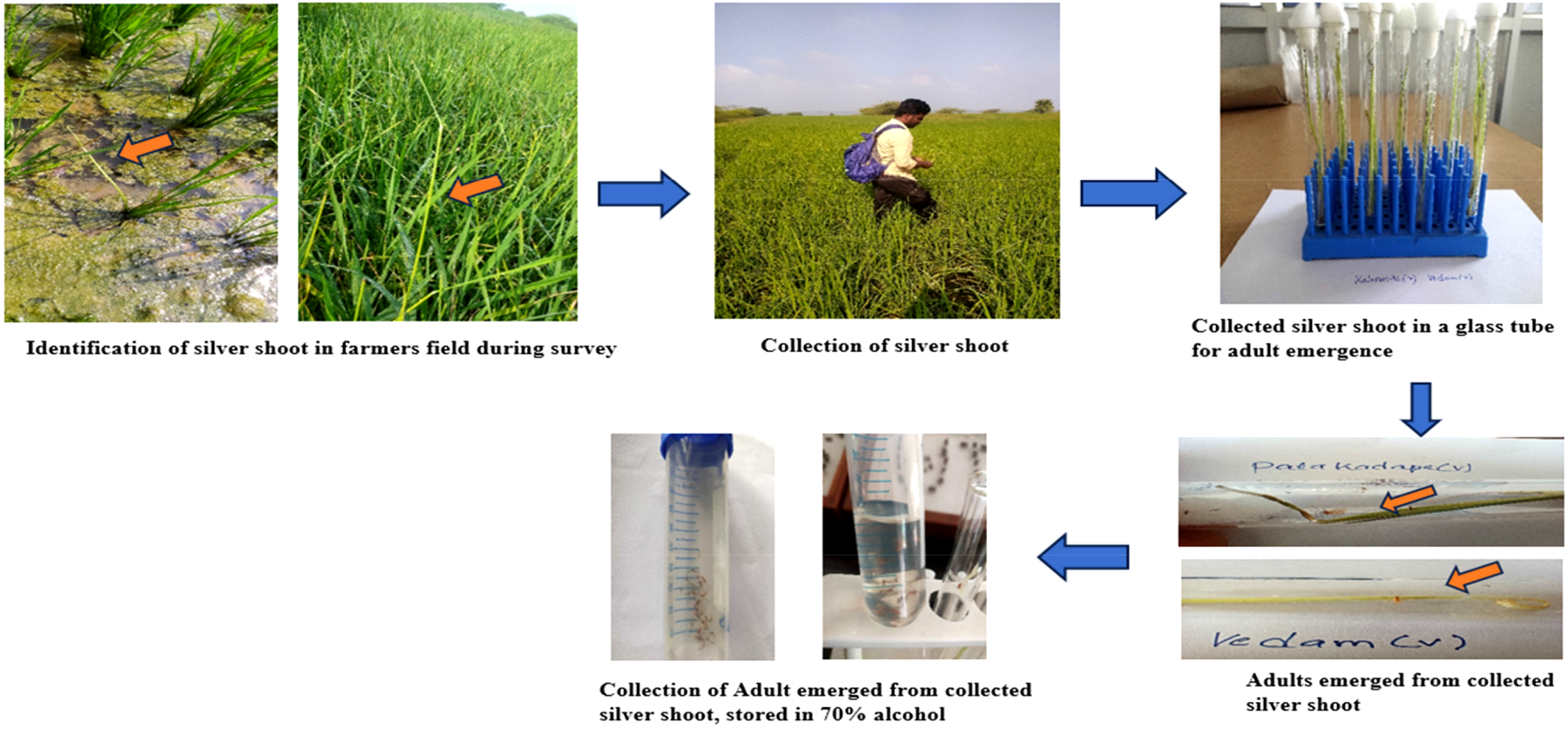


Plate 3.3: Identification, collection of silver shoot and storage of emerged adults in 70% alcohol during survey for the incidence of rice gall midge, *Orseolia oryzae* in Southern zone of Andhra Pradesh

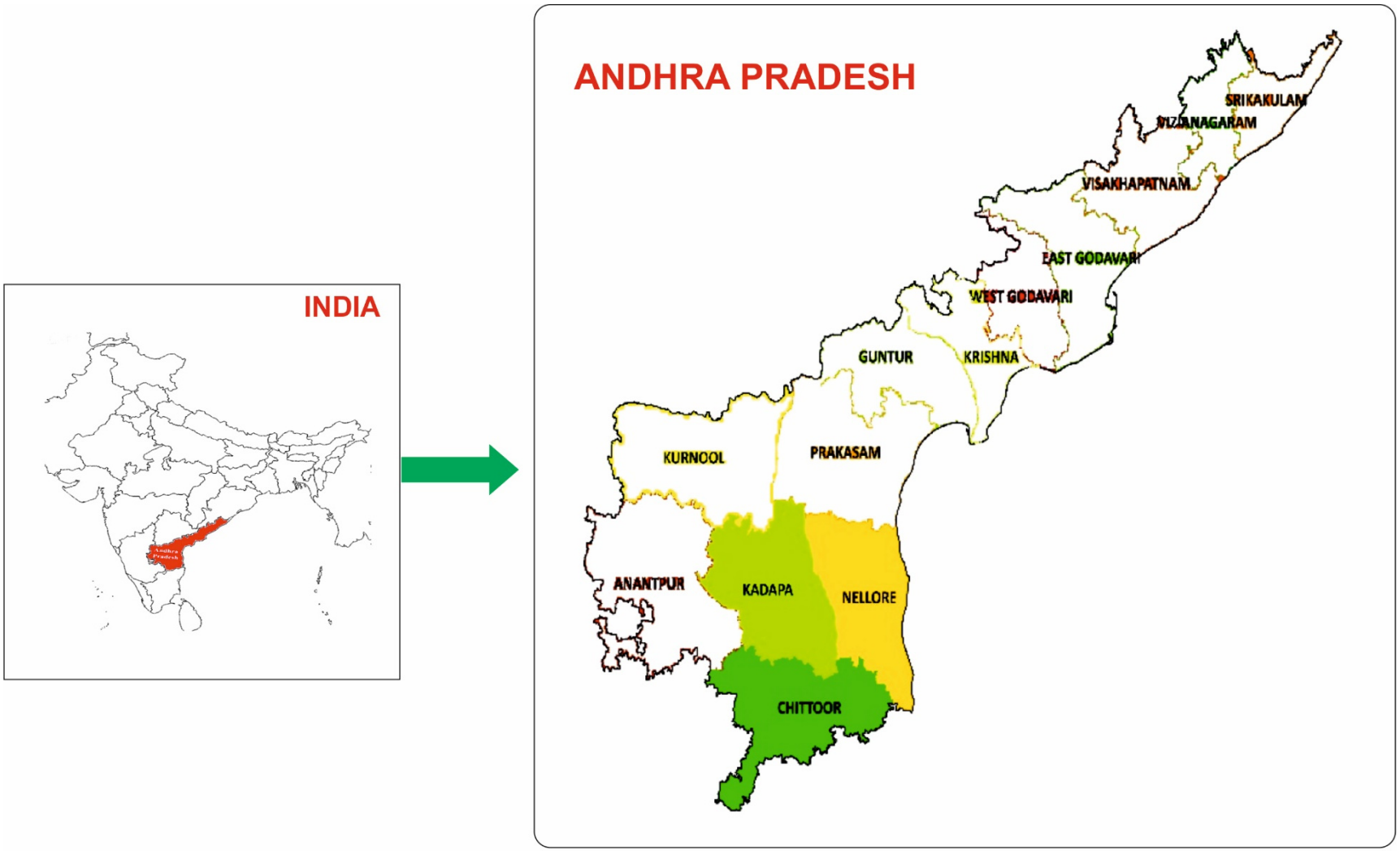


Fig 3.1: Survey map showing collection sites of rice gall midge insects from different Districts of Andhra Pradesh

Table 3.1 List of Districts, mandals and villages of Southern zone of Andhra Pradesh surveyed for the incidence of rice gall midge during *kharif/ rabi*, 2022.

District	Mandal	Village	Farmer No.	Latitude
Chittoor	Sri kalahasti	Cherlopalle	1	13.7253°N, 79.6610°E
			2	13.7262°N, 79.6637°E
			3	13.7262°N, 79.6634°E
			4	13.7266°N, 79.6640°E
			5	13.7267°N, 79.6631°E
		Vedam	1	13.6675°N, 79.6872°E
			2	13.6677°N, 79.6874°E
			3	13.6677°N, 79.6860°E
			4	13.6681°N, 79.6868°E
			5	13.6683°N, 79.6879°E
	Yerpedu	Anjimedu	1	13.6750°N, 79.5787°E
			2	13.6753°N, 79.5789°E
			3	13.6758°N, 79.5792°E
			4	13.6747°N, 79.5787°E
			5	13.6750°N, 79.5779°E
		Merlapaka	1	13.7031°N, 79.6139°E
			2	13.7037°N, 79.6144°E
			3	13.6996°N, 79.6171°E
			4	13.7042°N, 79.6156°E
			5	13.7039°N, 79.6161°E
	Tirupati Rural	Peruru	1	13.5755°N, 79.3096°E
			2	13.5766°N, 79.3108°E
			3	13.5756°N, 79.3129°E
			4	13.5773°N, 79.3104°E
			5	13.5749°N, 79.3084°E
		Cherlopalli	1	13.6094°N, 79.3665°E
			2	13.6117°N, 79.3660°E
			3	13.6121°N, 79.3652°E
			4	13.6118°N, 79.3653°E
			5	13.6114°N, 79.3660°E
Kadapa	Kadapa Rural	Kotha Nellore	1	14.4370°N, 78.8052°E
			2	14.4368°N, 78.8053°E
			3	14.4368°N, 78.8054°E
			4	14.4367°N, 78.8057°E
			5	14.4373°N, 78.8076°E
		Patha Kadapa	1	14.4986°N, 78.8369°E
			2	14.4989°N, 78.8362°E
			3	14.4994°N, 78.8377°E
			4	14.4975°N, 78.8377°E
			5	14.4976°N, 78.8386°E
	Vallur	Cheruvukindapalli	1	14.5945°N, 78.7028°E
			2	14.5948°N, 78.7021°E
			3	14.5935°N, 78.6986°E

			4	14.5937°N, 78.7050°E
			5	14.5968°N, 78.7004°E
		Chinna lebaka	1	14.5397°N, 78.6854°E
			2	14.5381°N, 78.6854°E
			3	14.5438°N, 78.6852°E
			4	14.5426°N, 78.6888°E
			5	14.5417°N, 78.6930°E
	Khajipet	Ravulapalle	1	14.6369°N, 78.7705°E
			2	14.6358°N, 78.7697°E
			3	14.6365°N, 78.7690°E
			4	14.6395°N, 78.7796°E
			5	14.6408°N, 78.7804°E
		Chemullapalle	1	14.6198°N, 78.7972°E
			2	14.6221°N, 78.7984°E
			3	14.6196°N, 78.7960°E
			4	14.6159°N, 78.7969°E
			5	14.6203°N, 78.7981°E
Nellore	Vidavalur	Gadeladhinne	1	14.6294°N, 80.0974°E
			2	14.6301°N, 80.0972°E
			3	14.6302°N, 80.0977°E
			4	14.6287°N, 80.0975°E
			5	14.6298°N, 80.0984°E
		Dandigunta	1	14.6184°N, 80.1016°E
			2	14.6164°N, 80.1030°E
			3	14.6167°N, 80.1021°E
			4	14.6165°N, 80.1016°E
			5	14.6187°N, 80.1017°E
	Chittamur	Buradagali	1	13.9563°N, 80.0648°E
			2	13.9556°N, 80.0649°E
			3	13.9563°N, 80.0644°E
			4	13.9547°N, 80.0647°E
			5	13.9562°N, 80.0645°E
		Kothapalem	1	14.0689°N, 80.0488°E
			2	14.0694°N, 80.0486°E
			3	14.0686°N, 80.0494°E
			4	14.0696°N, 80.0487°E
			5	14.0688°N, 80.0496°E
	Sullurupeta	Illupuru	1	13.7176°N, 79.9770°E
			2	13.7183°N, 79.9770°E
			3	13.7167°N, 79.9779°E
			4	13.7197°N, 79.9768°E
			5	13.7180°N, 79.9740°E
		Vellukadu	1	13.7959°N, 80.0764°E
			2	13.7951°N, 80.0772°E
			3	13.7965°N, 80.0770°E
			4	13.7975°N, 80.0785°E
			5	13.7978°N, 80.0767°E

3.2 DETECTION OF RICE GALL MIDGE BIOTYPE UNDER FIELD CONDITIONS IN Y.S.R DISTRICT AND ITS MOLECULAR IDENTIFICATION

3.2.1 Detection of Rice Gall Midge Biotype under field conditions

3.2.1.1 Experimental site

Detection of rice gall midge biotype under field conditions was carried out with 17 standard differentials received from Agricultural Research Station (ARS), Ragolu and 16 differentials from Indian Institute of Rice Research (IIRR), Hyderabad experiment was conducted at Agricultural Research Station (ARS), Utukur, Kadapa, Y.S.R District (Table 3.3 and 3.4) (Plate 3.4).

3.2.1.2 Cultural Practices

All the agronomic practices were adopted as per the recommendation of ANGRAU in raising the crop except for management of pests and diseases practices during the experiment period. The field evaluation protocols developed by Kalode and Bentur (1989) was followed.

3.2.1.3 Preparatory Cultivation

The experiment area was prepared by deep summer ploughing with tractor and levelled before transplanting. The field was prepared by ploughing and harrowing for fine tilth during late *kharif* 2022-23.

3.2.1.4 Nursery raising and transplantation of seedling in main field

Each differential was grown in a separate nursery bed in a raised nursery bed method and labelled with tag. The experiment was laid out in a randomised block design (RBD) with 17 differentials provided by Agricultural Research Station, Ragolu and 16 differentials provided by IIRR, Hyderabad which are replicated twice. Manual transplantation was done after approximately 28-30 days of sowing. While transplanting, single seedling per hill was transplanted in a single row of 20 hills with a spacing of 20 cm between the rows and 15 cm within the rows.

a. Field view of Experimental plot for 17 differentials of rice gall midge at first date of transplantation during late *kharif* 2022

b. Field view of Experimental plot for 17 differentials of rice gall midge at second date of transplantation during late *kharif* 2022

Plate 3.4 (a and b): Overall field views of Experimental plot for 17 differentials of rice gall midge at different dates of transplantation during late *kharif* 2022.

3.2.1.5 Fertilizer Application

Except for Nitrogen recommended dose of fertilizers were applied, more nitrogen was top-dressed to get higher infestation of gall midge.

Table 3.2 Experimental Details

Locations	Differentials from	Date of Sowing Nursery	Date of Transplanting
Utukur	ARS, Ragolu	10-07-2022	08-08-2022
Utukur	ARS, Ragolu	25-08-2022	23-09-2022
Utukur	IIRR, Hyderabad	12-09-2022	13-10-2022

3.2.1.6 Method of Observations

During the period of study, the incidence of rice gall midge in term of per cent silver shoot and plant damage in different rice differentials were recorded at 30 and 50 DAT.

Observations to be recorded at 30 and 50 DAT:

Per cent of damaged plants and the per cent of damaged tillers were recorded. Of the two data sets recorded on 30 and 50 DAT, the set showing higher damage in the susceptible check was considered valid.

When a particular biotype is not following any specific reaction pattern of an identified biotype, percent similarity index (PSI) aids in comparing the similarity of either resistant (R) or susceptible (S) reaction of an existing biotype at a location to the reaction pattern of the identified biotypes. The reaction pattern shown by a biotype of a location similar to the identified biotype can be known with this value.

The Per cent Similarity Index (PSI) was calculated by using the formula

$$PSI=\frac{\text{Total no. of differentials - Total no. of dissimilar reactions}}{\text{Total no. of differentials}}\times 100$$

Table 3.3 List of 17 differentials used for rice gall midge experiment from ARS Ragolu.

Group	Entry No.	Differential
I	1	KAVYA
	2	W 1263
	3	ARC 6605
II	4	PHALGUNA
	5	ARC 5984
	6	DUKONG – 1
	7	RP-2333-156-8
	8	MADHURI L 9
	9	BG-380-2
III	10	MR 1523
IV	11	RP 2068-18-3-5
	12	ABHAYA
	13	INRC 3021
	14	AGANNI
	15	INRC 15888
	16	B 95-1
V	17	TN-1

Table 3.4 List of 16 differentials used for rice gall midge experiment from IIRR Hyderabad.

Group	Entry No.	Differential
I	1	KAVYA
	2	W 1263
	3	ARC 6605
II	4	PHALGUNA
	5	ARC 5984
	6	DUKONG – 1
	7	RP-2333-156-8
	8	MADHURI L 9
	9	BG-380-2
III	10	MR 1523
IV	11	RP 2068-18-3-5
	12	ABHAYA
	13	INRC 3021
	14	AGANNI
	15	INRC 15888
V	16	TN-1

3.2.2 Molecular Identification of Rice Gall Midge Biotype

3.2.2.1 Collection of Insects

Plants infested with rice gall midge were collected from farmer's fields during the survey. Generally, the adults emerge in the next day evening, adults were collected and stored in 70% ethanol and preserved at -20ºC till DNA extraction.

3.2.2.2 Location

Insects were collected from Chittoor, Y.S.R and Nellore districts of Andhra Pradesh, India.

3.2.2.3 Extraction procedure

- Genomic DNA from single female insect was extracted following Behura *et. al.* (1999) and Atray *et al.* (2015) with modification.
- The insects collected were stored in 70% ethyl alcohol. The wings and legs were removed before processing for DNA extraction. Each insect was soaked in 50 µl of extraction buffer for 10 minutes in 1.5 ml eppendorf tube.
- The insect was homogenized in 1.5 ml eppendorf tube with sterilised polypropylene micro pestle gently and thoroughly.
- Once again 350 µl of extraction buffer was added slowly by rinsing the pestle.
- 16 µl of Proteinase K was added and incubated at 37°C for 1 hour.
- Add 400 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the tube, inverted gently and spinned at 14,000 rpm at 25°C for 5 minutes.
- The supernatant was transferred to a new tube with a pipette avoiding protein and debris layer.
- Add 400 µl of chloroform: isoamylalcohol (24:1) was added, inverted gently and spinned at 14,000 rpm at 25°C for 5 minutes.
- The supernatant was transferred to a new tube and 20 µl of RNase A was added to each tube.

- Incubated in water bath for 1 hours at 37°C.
- Add 400 µl phenol: chloroform: isoamyl alcohol (25:24:1) mixed gently and spinned at 14,000 rpm at 25°C for 5 minutes.
- The supernatant was transferred to a new tube, followed by addition of 400 µl of chloroform: isoamylalcohol (24:1) and spinned at 14,000 rpm at 25°C for 5 minutes.
- The supernatant was transferred to a new tube and mixed with 16µl of 0.2M NaCl.
- Add twice volume ice-cold absolute ethanol, shake gently and left overnight at -20°C.
- Next day, the tube was spinned at 13,000 rpm at 4°C for 20-30 minutes.
- The supernatant was removed with pipette, taking care not to dislocate the DNA pellet.
- The DNA pellet was washed with 200µl 70% alcohol, air dried for 15 minutes.
- Finally, DNA pellet was dissolved in 20µl TE.
- Then 1µl of the genomic DNA was electrophoresed in 1.0 % agarose gel in 1X TBE Buffer to check the quality and yield of the same (Appendix-II).

3.2.2.5 Quality and Quantity Check of DNA Isolated

The purity and concentration of isolated genomic DNA were estimated by agarose gel electrophoresis (1.0% agarose) and by using Nanodrop Spectrophotometer.

Agarose gel electrophoresis was carried out to check the presence of DNA. After the gel run, the gel was visualized in a UV light transmitted gel documentation system. After checking the concentration of the DNA based on the band intensity, and also based on the readings from the nanodrop spectrophotometer the DNA samples were diluted (50 ng/µl) for further Polymerase Chain Reaction (PCR) analysis (Plate 3.5).

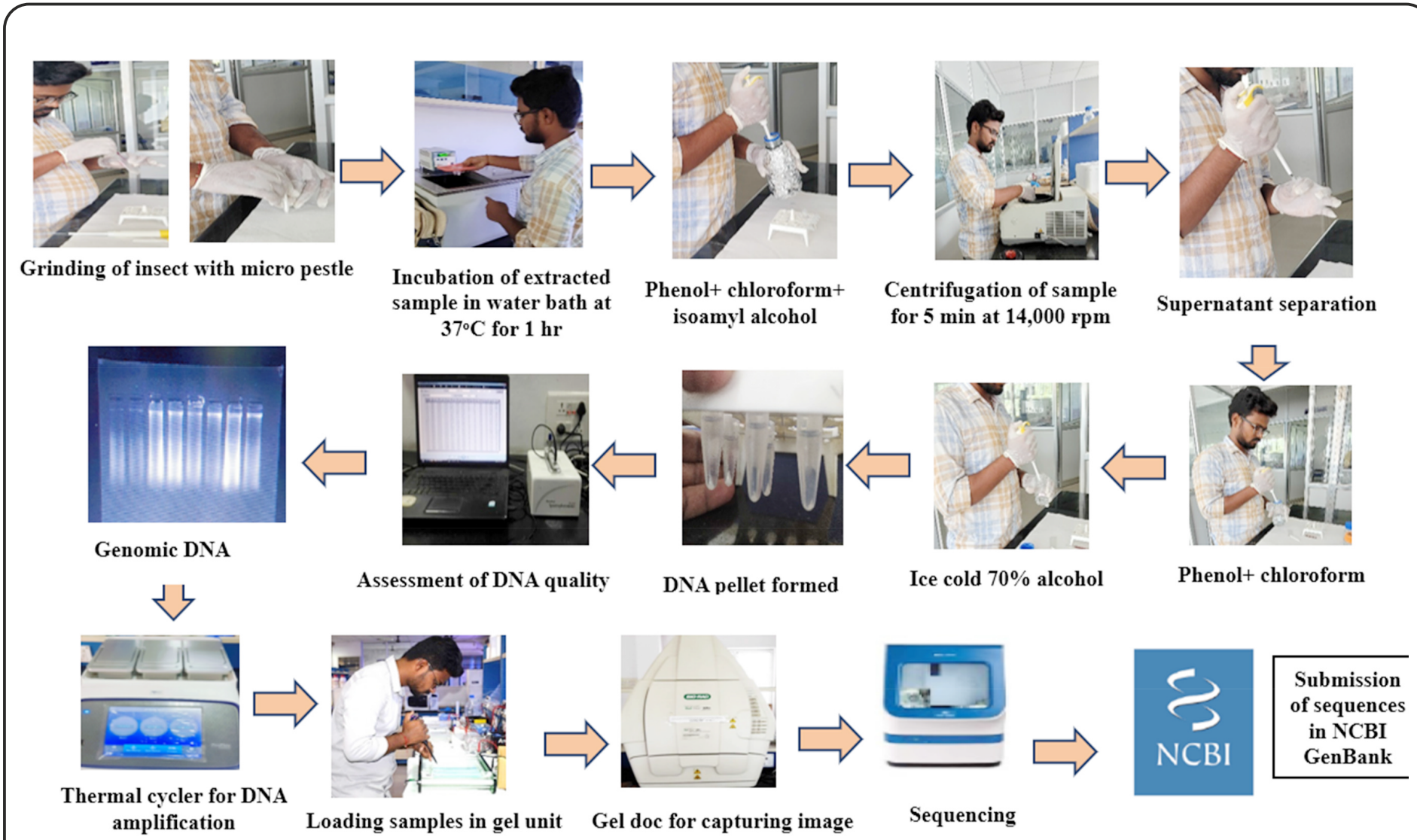


Plate 3.5: Molecular characterization studies with rice gall midge, *Orseolia oryzae*

Table 3.5: List of primers/ markers

S.No	Primers/ markers	Locus/ Oligo name	Forward and backward sequences
1	SSR	Oosat16	F: TGTTCAGCTTGTTCAGC R: CATTGGAACGAAATTAGTGG
2	SSR	Oosat21	F: CCGATTTCACTCGATGTTGTT R: TTCTAACTTGAACTCCTCATTCG
3	SSR	Oosat24	F: CCTCGGTCGCATCTCATATT R: CCATTCAACAGATTTGGCGTA
4	SSR	Oosat35	F: GCCCGTTGATTGCTTTGTAT R: TATCGTTGTCGTCGTCGTCTTCG
5	SSR	Oosat43	F: TCGTTGGAATAGCACATTCG R: TGACGTGTCTATGCCATGTG
6	SSR	Oosat59	F: CGTCGCCTTGTTTAATATG R: CCAATTGTGTTGCTTGA
7	SCAR	Y132	F: AGAAATCGATTCCAGGACGT R: TTAGCCCGATAAATCTTTCAC
8	SCAR	Y133	F: ATGGTTTTACATTAAGATGAAAT R: ATTTTACCAGAATCGCGATG
9	mtCOI	-	F: CATTGGAGATGACCAAATTTATAATG R: TAAACTTCAGGGTGACCAAAAAATCA

3.2.2.6 Polymerase Chain Reaction

The genomic DNA of gall midge collected from different locations in Andhra Pradesh was subjected to PCR amplification. PCR was carried out using a programmable thermocycler. The PCR conditions used are as follows:

PCR Conditions for SSRs, SCARs and mtCOI Primers

S. No.	Steps	Temperature	Time
1	Initial Denaturation	94ºC	2 Min
2	Cycle Denaturation	94ºC	1 Min
3	Annealing	55-61ºC	1 Min
4	Extension	72ºC	2 Min
5	Final Extension	72ºC	7 Min
6	Cooling	4ºC	α
30 cycles			

PCR Components:

Taq buffer (10X)	2.5 µl
$MgCl_2$ (2.5mM)	2.0 µl
d NTPS (10 mM)	0.5 µl
Template DNA (50ng/µl)	4.0 µl
Forward Primer (10pM)	1.0 µl
Reverse Primer (10pM)	1.0 µl
Taq polymerase (5U/µl)	0.3 µl
Nuclease free water	13.7 µl
Total	25.0 µl

3.2.2.7 Agarose Gel Electrophoresis

- About 3g (3% gel) of Agarose was dissolved in 100 ml of 1.0 X TBE buffer and was melted in a microwave oven.
- To the melted Agarose, about 4 µl of ethidium bromide solution (10 mg/ml) was added.
- The melted Agarose was then poured into a gel cast fixed with appropriate gel combs (0.5mm) without formation of bubbles and was allowed to solidify at room temperature for 45 min to 1 hr.
- After gel casting, the gel was transferred into a horizontal electrophoresis unit containing 1.0 X TBE buffer solution.
- Before loading the PCR product in the wells of Agarose gel; the PCR amplified product (2 µl) were mixed with 2 µl of loading dye.
- Then the mixture (PCR product + loading dye) was loaded into each well of the gel along with 100 bp DNA ladder in the first well and run at a constant voltage of 150 volts for 2.0 hr.

3.2.2.8 Gel Documentation

After completion of the gel run, the gels were visualized in a UV gel documentation system (Bio-Rad Laboratories, Inc., Berkeley, California) for

analysis of DNA bands. The banding pattern was observed and recorded in the gel documentation unit. The size of the amplified fragments was calculated using Bio-Rad Gel documentation system (Bio-Rad Laboratories, Inc., Berkeley, Californa) with 100 bp DNA ladder (3422A) as size reference standard.

3.2.2.9 DNA sequencing

The amplified product was sequenced by using the PCR purification at an DNA sequencing facility (Eurofins Genomics India Pvt Ltd., Bengaluru) sequence assembling and nucleotide alignment were done with Bio Edit version 7.0 software. The nucleotide sequences are compared with rice gall midge sequences of all the seven biotypes of India, sequences were collected from NCBI GenBank database (NCBI, http://www.ncbi.nlm.nih.org) and the construction of phylogenetic tree (Maximum Likelihood method and bootstrap option) was done using MEGA 11.0 software.

3.3 SCREENING OF RICE GENOTYPES AGAINST RICE GALL MIDGE IN Y.S.R. DISTRICT.

3.3.1 Experimental site

Screening of 50 rice genotypes (including two checks) against rice gall midge experiment was conducted at Agricultural Research Station (ARS), Utukur, Kadapa, Y.S.R District, during late *kharif* 2022 (Fig 3.2).

3.3.1 Cultural Practices

All the agronomic practices were adopted as per the recommendation of ANGRAU in raising the crop and no pest management practices were carried out during the experimentation. The field evaluation protocols developed by Kalode and Bentur (1989) were followed.

3.3.2 Preparatory Cultivation

The experiment area was prepared by deep summer ploughing with tractor and levelled before transplantation. The field was prepared by ploughing and harrowing for fine tilth during late *kharif* 2022-23.

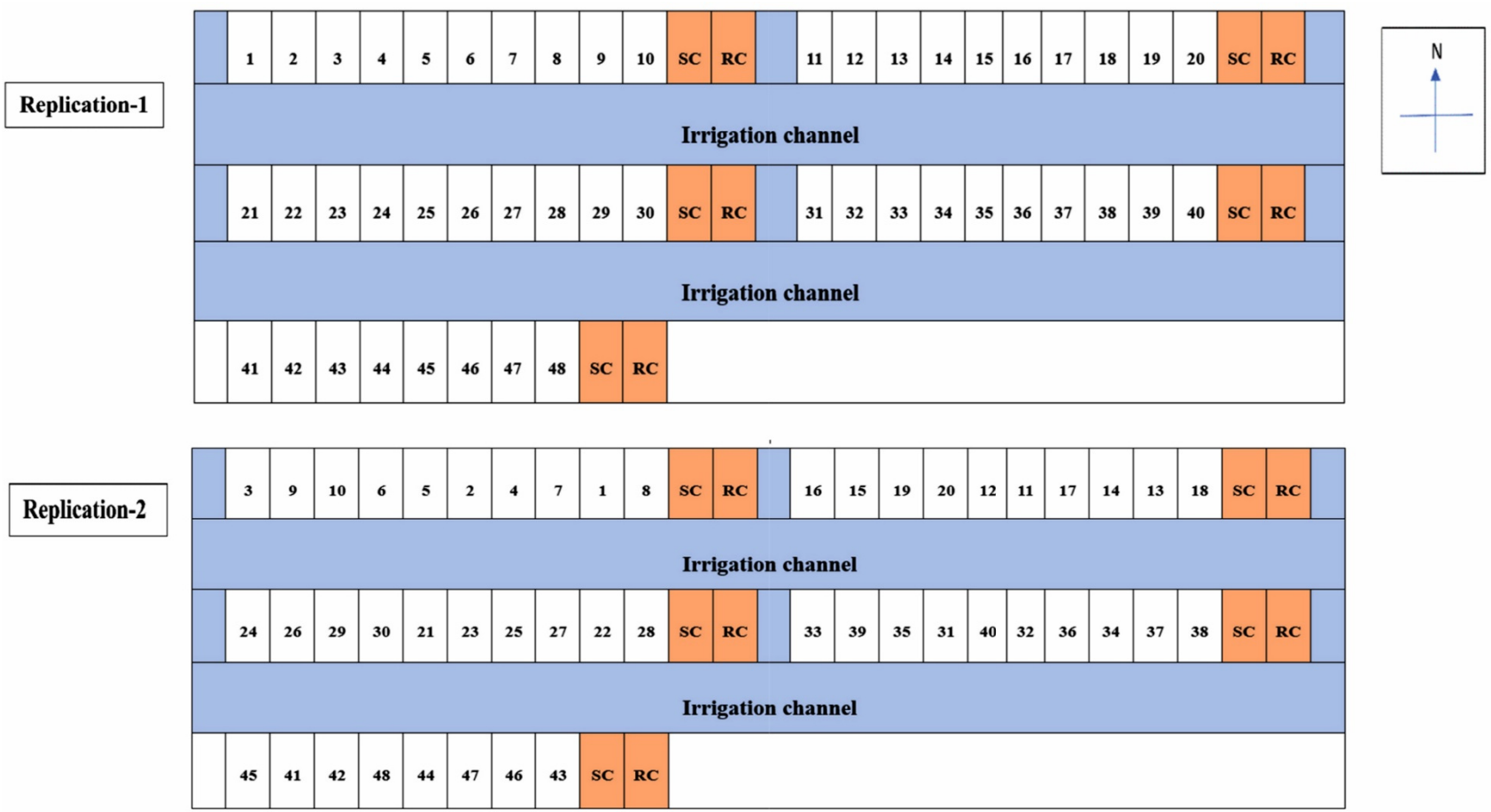


Fig. 3.2 Field layout of rice genotypes for screening experiment (1-50 entries number, SC- Susceptible Check and RC - Resistant Check)

3.3.3 Nursery raising and transplantation of seedling in main field

Each genotype was sown in raised nursery bed. The experiment was laid out in a randomised block design (RBD) with 50 genotypes (includes two checks) provided by Agricultural Research Station (ARS), Ragolu, which were replicated twice. Manual transplantation was done at 28 days after nursery sowing. Each genotype was transplanted in a single row of 33 hills per genotype with a spacing of 20 cm between the rows and 15 cm within the rows and used single seedling per hill. For every 10 genotypes, single row of each resistant check PTB-33 and susceptible check TN-1 were transplanted.

3.3.4 Fertilizer Application

Except for Nitrogen recommended dose of fertilizers were applied, more nitrogen was top-dressed to get higher infestation of gall midge.

3.3.5 Experimental Material

48 rice genotypes, one resistant check PTB-33 and one susceptible check TN-1 were received from Agricultural Research Station (ARS), Ragolu. (Table 3.6 and plate 3.6 and 3.7).

3.3.6 Method of Observations

During the period of study, the incidence of rice gall midge in term of per cent silver shoot and plant damage in different rice genotypes were recorded at 30 and 50 DAT.

Observations to be recorded at 30 and 50 DAT:

1. Observations on total plants and damaged plants due to RGM were recorded.
2. The number of total tillers and silver shoots in a maximum of 10 plants per genotype were recorded.

$$\% \text{ Damaged plants} = \frac{\text{Total number of infested plants}}{\text{Total number of plants}} \times 100$$

$$\% \text{ Silver shoots} = \frac{\text{Total numberof tillers with silver shoots}}{\text{Total number of tillers}} \times 100$$

3.3.7 Groups of genotypes based on rice gall midge incidence

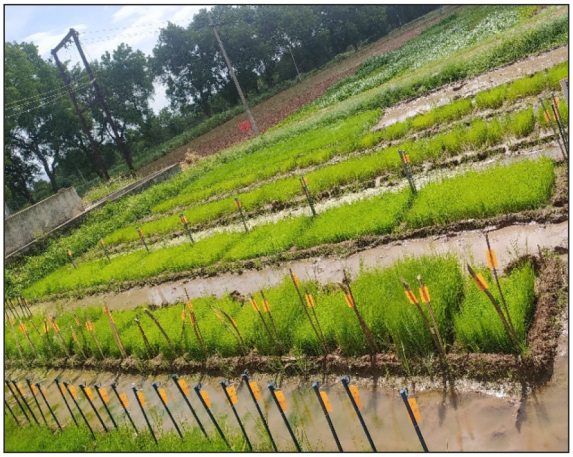
Resistance or susceptibility of genotypes were categorized based on per cent gall midge incidence, following a six-point (0-9) rating scale, which was provided by IRRI 2002 (Table 3.7).

Table 3.7 Rating scale based on per cent silver shoot incidence by IRRI 2002

Scale	Infected tillers in field Test	Reaction
0	No damage	Highly Resistant
1	Less than 1%	Resistant
3	1-5%	Moderately Resistant
5	6-10%	Moderately Susceptible
7	11-25%	Susceptible
9	More than 25%	Highly Susceptible

3.3.7 Statistical Analysis

The data regarding per cent silver shoot and per cent plant damage per genotype at 30 and 50 DAT were subjected to arcsine transformation and statistical analysis was done using ANOVA (Analysis of Variance) with help of SPSS 20.0 statistical package.




(a) Nursery raising for rice genotypes in screening trial


(b) Main field layout for rice genotypes in screening trial

Plate 3.6: (a and b) Rice genotypes for rice gall midge screening trial experiment

(a) Data collection at 30 and 50 DAT rice genotypes in screening experiment

(b) Overview of a rice genotypes in screening experiment

Plate 3.7 (a and b) Main field layout for rice genotypes in screening trial

Table 3.6 List of 50 rice genotypes used for screening experiment

S. No	Genotype	Designation	S. No	Genotype	Designation	S. No	Genotype	Designation
1	DG-130	RP 4613-261	19	DG-165	RPE 1042	37	DG-191	RPE 1464
2	DG-131	RP 4613-263	20	DG-167	RPE 1183	38	DG-192	RPE 1564
3	DG-137	RP 4621 -1845	21	DG-168	RPE 1205	39	DG-198	RP 4621-1845
4	DG-144	RPE 733	22	DG-170	RPE 1245	40	DG-200	RP 4639-179
5	DG-145	RPE 735	23	DG-171	RPE 1249	41	DG-201	RP 4639-336
6	DG-148	RPE 739	24	DG-173	RPE 1257	42	DG-202	RP 4639-461
7	DG-151	RPE 745	25	DG-174	RPE 1258	43	DG-204	RP 4643-1025
8	DG-153	RPE 920	26	DG-176	RPE 1261	44	DG-211	GEMP-548
9	DG-154	RPE 921	27	DG-178	RPE 69	45	DG-212	GEMP-602
10	DG-155	RPE 924	28	DG-180	RPE 99	46	DG-217	RP 4092-365-117-10
11	DG-156	RPE 928	29	DG-181	RPE 177	47	DG-218	-
12	DG-157	RPE 937	30	DG-182	RPE 218	48	DG-221	RP 4680-1-1-15
13	DG-158	RPE 938	31	DG-183	RPE 220	49	TN-1	-
14	DG-159	RPE 939	32	DG-184	RPE 259	50	PTB-33	-
15	DG-160	RPE 940	33	DG-185	RPE 272			
16	DG-162	RPE 1031	34	DG-187	RPE 1154			
17	DG-163	RPE 1034	35	DG-189	RPE 1385			
18	DG-164	RPE 1039	36	DG-190	RPE 1387			

Chapter - IV

Results & Discussion

Chapter - IV

RESULTS AND DISCUSSION

The present investigations on the "**Survey, Varietal Screening and Molecular identification of Asian Rice Gall Midge in Y.S.R District of Andhra Pradesh"** carried out at Agricultural Research Station, Utukur, Kadapa, Y.S.R District and Regional Agricultural Research Station, Tirupati, Chittoor District during 2022-2023. The results pertaining to the study were presented here under and discussed with allied literature.

4.1 SURVEY FOR THE INCIDENCE OF RICE GALL MIDGE ACROSS THE SOUTHERN ZONE OF ANDHRA PRADESH.

Roving survey were conducted in major rice growing districts of Southern zone of Andhra Pradesh *viz*., Chittoor, Nellore and Y.S.R districts to record the per cent plant damage and per cent silver shoot of Rice Gall Midge (RGM) during *kharif,* 2022 in Y.S.R District and *rabi,* 2022 in Chittoor and Nellore Districts. The collected data on RGM infestation were presented as mean plant damage (%) and mean silver shoot (%) (Table 4.1). Among the three districts surveyed for RGM infestation, Nellore District recorded highest mean silver shoot 15.38 per cent followed by Y.S.R District of 12.19 per cent and lowest recorded in Chittoor District about 4.50 per cent. Highest mean plant damage 76.00 % was recorded in Y.S.R District followed by Nellore District (71.20 %) and Chittoor District (49.92 %) (Table 4.3) (Fig. 4.2).

4.1.1 Incidence of rice gall midge, *O. oryzae* in different mandals and villages of Chittoor District of Andhra Pradesh during *rabi*, 2022-23.

In Chittoor District, highest per cent mean silver shoot damage due to RGM of 6.88 per cent recorded in Sri kalahasti mandal, medium damage in Yerpedu mandal (3.56%) and lowest of 3.06 per cent in Tirupati Rural. Among the different villages surveyed, the per cent mean plant damage and silver shoot damage of RGM was 40.50, 75.00 and 3.45, 10.30 per cent in Cherlopalle, Vedam villages of Sri kalahasti mandal, respectively. However, the per cent mean plant damage and silver shoot damage of RGM was 39.00, 55.00 and 2.69, 4.42 per cent in

Anjimedu, Merlapaka villages of Yerpedu mandal, respectively. In Peruru, Cherlopalli villages of Tirupati Rural, the per cent mean plant damage of 45.00 and 45.00 per cent and silver shoot damage of 2.67, 3.45 per cent was recorded respectively (Table 4.1).

4.1.2 Incidence of rice gall midge, *O. oryzae* in different mandals and villages of Y.S.R District of Andhra Pradesh during *kharif*, 2022-23.

In Y.S.R District, highest per cent mean silver shoot damage due to RGM of 14.35 per cent was recorded in Vallur mandal, medium damage in Khajipet mandal (12.22%) and lowest of 10.02 per cent in Kadapa Rural. The per cent mean plant damage and silver shoot damage of RGM was 72.00, 81.00 and 14.16, 14.53 per cent in Cheruvukindipalli and Chinna lebaka villages of Vallur mandal respectively. However, in Khajipet mandal, the per cent mean plant damage and silver shoot damage of RGM was 98.00, 77.00 and 13.04, 11.39 per cent in Ravulapalle and Chemullapalle villages, respectively. Kotha Nellore, Patha Kadapa villages of Kadapa Rural, recorded the RGM per cent mean plant damage and silver shoot damage of 60.00, 68.00 and 8.78, 11.25 per cent (Table 4.1).

4.1.3 Incidence of rice gall midge, *O. oryzae* in different mandals and villages of Nellore District of Andhra Pradesh during *rabi*, 2022-23.

In Nellore District, highest per cent mean silver shoot damage due to RGM of 25.91 per cent in Sullurupeta mandal, medium damage in Vidavalur mandal (10.88%) and lowest of 9.41 per cent recorded in Chittamar mandal. The per cent mean plant damage and silver shoot damage of RGM was 100.00, 53.00 and 45.30, 6.50 in Vellukadu, Illupuru villages of Sullurupeta mandal, respectively. However, in Vidavalur mandal of Nellore District, the mean per cent plant damage and silver shoot damage was 74.00, 67.00 and 17.65, 4.00 in Gadeladhinne, Dandigunta villages respectively. In Buradagali and Kothapalem villages of Chittamur mandal, the mean per cent plant damage and silver shoot damage of 79.00, 54.00 and 12.94, 5.87 per cent was recorded respectively (Table 4.1).

The per cent mean silver shoot and plant damage of RGM in different surveyed mandals were presented in Table 4.2. Among all the mandals of three districts surveyed, the highest per cent mean silver shoot and plant damage caused

by RGM were recorded in Sullurupeta mandal (25.91%) of Nellore District and Khajipet mandal (87.50%) of Y.S.R District. Lowest per cent mean silver shoot and plant damage of 3.06 and 45.00 per cent were recorded in Tirupati Rural of Chittoor District respectively (Fig 4.1).

During the survey, the highest per cent plant damage (100.00%) and silver shoot damage (45.30%) of RGM was recorded in Vellukadu village of Sullurupeta mandal in Nellore District while the lowest mean per cent plant damage (45.00%) was recorded in Anjimedu village and mean per cent silver shoot damage (2.67%) was recorded in Peruru village in Chittoor District of Andhra Pradesh.

The low incidence in Chittoor District was may be due to abiotic factors are not favorable to the rice gall midge, high Granular application and insecticidal sprays from the nursery itself which are followed by most of the rice farmers for the management of RGM control compared to surveyed rice farmers of Nellore and Y.S.R Districts of Andhra Pradesh.

Most of the farmers of Y.S.R District are using the insecticides such as Acephate and Chlorpyriphos and Carbofuran 3G granular application at tillering stage of rice crop.

The farmers of Nellore District, mostly Vellukadu village were not much aware of latest chemicals and mostly they were using the conventional insecticides like neem formulations which is not effective against RGM and village Gadeladhinne, an NLR-34449 variety as high incidence of RGM was noticed compared to other varieties may be due to the late transplantation and favorable environmental conditions for RGM.

The above survey on rice gall midge incidence were in similar with the per cent silver shoot damage studies conducted by Prasad, (2011) who reported silver shoot damage range of 12.14 to 34.35 per cent in Jharkhand. Mamathad *et. al.* (2020) conducted survey on incidence of Asian rice gall midge and reported 6.95 to 9.03 per cent of mean silver shoot damage in Cauvery and Kabini command area, Karnataka. Surendra and Bindhu (2016), conducted survey on major insect pests of rice crop in Patna District of Bihar and observed silver shoot damage of 6.66 to 12.50 per cent at vegetative stage of rice crop during *kharif*, 2013-14.

Table 4.1 Incidence of rice gall midge, *Orseolia oryzae* on rice in different rice growing districts, mandals and villages Southern zone of Andhra Pradesh during *kharif/ rabi* 2022.

DISTRICT	MANDAL	VILLAGE	FARMER NO.	LATITUDE	VARIETY	DAP	TP	TT	Mean %TT	SS	Mean SS	%SS	Mean %SS	%PD	Mean %PD
Chittoor	Sri kalahasti	Cherlopalle	1	13.7253°N, 79.6610°E	BPT-5204	40	20	275	294	17	10.4	6.18	3.45	60	40.5
			2	13.7262°N, 79.6637°E	NDLR-7	45	20	349		14		4.01		57.5	
			3	13.7262°N, 79.6634°E	RNR-15048	25	20	253		5		1.98		20	
			4	13.7266°N, 79.6640°E	RNR-15048	25	20	264		3		1.14		15	
			5	13.7267°N, 79.6631°E	NDLR-7	30	20	329		13		3.95		50	
		Vedam	1	13.6675°N, 79.6872°E	ADT-37	60	20	300	351.8	50	33.8	16.67	10.30	85	75
			2	13.6677°N, 79.6874°E	ADT-37	60	20	282		43		15.25		75	
			3	13.6677°N, 79.6860°E	ADT-37	60	20	359		29		8.08		80	
			4	13.6681°N, 79.6868°E	ADT-37	60	20	416		23		5.53		70	
			5	13.6683°N, 79.6879°E	ADT-37	60	20	402		24		5.97		65	
	Yerpedu	Anjimedu	1	13.6750°N, 79.5787°E	BPT-5204	20	20	368	331.8	11	9	2.99	2.69	45	39
			2	13.6753°N, 79.5789°E	BPT-5204	20	20	338		7		2.07		30	
			3	13.6758°N, 79.5792°E	BPT-5204	25	20	323		12		3.72		50	
			4	13.6747°N, 79.5787°E	NDLR-7	20	20	338		10		2.96		45	
			5	13.6750°N, 79.5779°E	NDLR-7	30	20	292		5		1.71		25	

Contd…

		Merlapaka	1	13.7031°N, 79.6139°E	NDLR-7	55	20	277	375.8	6	17	2.17	4.42	30	55
			2	13.7037°N, 79.6144°E	NDLR-7	50	20	413		19		4.60		35	
			3	13.6996°N, 79.6171°E	BPT-5204	48	20	414		15		3.62		65	
			4	13.7042°N, 79.6156°E	BPT-5204	53	20	359		23		6.41		80	
			5	13.7039°N, 79.6161°E	NDLR-7	48	20	416		22		5.29		65	
	Tirupati rural	**Peruru**	1	13.5755°N, 79.3096°E	BPT-5204	45	20	413	397.4	9	10.6	2.18	2.67	40	45
			2	13.5766°N, 79.3108°E	BPT-5204	45	20	362		9		2.49		40	
			3	13.5756°N, 79.3129°E	ADT-37	53	20	403		12		2.98		50	
			4	13.5773°N, 79.3104°E	BPT-5204	55	20	407		11		2.70		50	
			5	13.5749°N, 79.3084°E	BPT-5204	55	20	402		12		2.99		45	
		Cherlopalli	1	13.6094°N, 79.3665°E	BPT-5204	48	20	292	386.4	5	13.8	1.71	3.45	25	45
			2	13.6117°N, 79.3660°E	ADT-37	62	20	413		19		4.60		35	
			3	13.6121°N, 79.3652°E	ADT-37	62	20	407		11		2.70		50	
			4	13.6118°N, 79.3653°E	BPT-5204	53	20	391		19		4.86		60	
			5	13.6114°N, 79.3660°E	BPT-5204	53	20	429		15		3.40		55	
Y.S.R	**Kadapa Rural**	**Kotha Nellore**	1	14.4370°N, 78.8052°E	JGL-384	34	20	402	456.4	44	39.8	10.94	8.78	65	60
			2	14.4368°N, 78.8053°E	NDLR-7	25	20	617		53		8.58		65	
			3	14.4368°N, 78.8054°E	NDLR-7	34	20	275		23		8.36		60	
			4	14.4367°N, 78.8057°E	JGL-384	42	20	536		42		7.83		60	

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			5	14.4373°N, 78.8076°E	JGL-384	21	20	452		37		8.18		50	
		Patha Kadapa	1	14.4986°N, 78.8369°E	JGL-384	15	20	451	517.2	47	58.4	10.45	11.25	75	68
			2	14.4989°N, 78.8362°E	BPT-5204	20	20	503		58		11.53		70	
			3	14.4994°N, 78.8377°E	BPT-5204	20	20	546		68		12.45		60	
			4	14.4975°N, 78.8377°E	BPT-5204	25	20	560		67		11.96		65	
			5	14.4976°N, 78.8386°E	JGL-384	20	20	526		52		9.88		70	
	Vallur	Cheruvukrindipalli	1	14.5945°N, 78.7028°E	BPT-5204	65	20	661	712.8	107	100.4	16.18	14.16	70	72
			2	14.5948°N, 78.7021°E	BPT-5204	65	20	785		90		11.46		70	
			3	14.5935°N, 78.6986°E	BPT-5204	65	20	775		112		14.54		70	
			4	14.5937°N, 78.7050°E	BPT-5204	65	20	680		106		15.48		70	
			5	14.5968°N, 78.7004°E	BPT-5204	65	20	663		87		13.12		80	
		Chinna lebaka	1	14.5397°N, 78.6854°E	BPT-5204	51	20	659	581.2	105	84	15.93	14.53	80	81
			2	14.5381°N, 78.6854°E	NDLR-7	55	20	582		97		16.67		85	
			3	14.5438°N, 78.6852°E	NDLR-7	55	20	550		78		14.88		85	
			4	14.5426°N, 78.6888°E	BPT-5204	48	20	569		67		11.78		75	
			5	14.5417°N, 78.6930°E	BPT-5204	48	20	546		73		13.37		80	
	Khajipet	Ravulapalle	1	14.6369°N, 78.7705°E	BPT-5204	62	20	645	674.8	87	89	13.48	13.04	100	98
			2	14.6358°N, 78.7697°E	NDLR-7	54	20	600		81		13.50		100	
			3	14.6365°N, 78.7690°E	NDLR-7	54	20	758		97		12.79		100	

Contd…

			4	14.6395°N, 78.7796°E	BPT-5204	60	20	579		58		10.01		90	
			5	14.6408°N, 78.7804°E	NDLR-7	50	20	792		122		15.40		100	
		Chemullapalle	1	14.6198°N, 78.7972°E	BPT-5204	45	20	755	759.8	78	86.6	10.33	11.39	80	77
			2	14.6221°N, 78.7984°E	NDLR-7	45	20	781		95		12.16		85	
			3	14.6196°N, 78.7960°E	BPT-5204	45	20	740		88		11.89		75	
			4	14.6159°N, 78.7969°E	BPT-5204	45	20	768		82		10.67		75	
			5	14.6203°N, 78.7981°E	BPT-5204	45	20	755		90		11.92		70	
Nellore	**Vidavalur**	**Gadeladhinne**	1	14.6294°N, 80.0974°E	NLR-34449	60	20	542	548.8	207	100.2	38.19	17.65	100	74
			2	14.6301°N, 80.0972°E	NLR-34449	60	20	454		117		21.46		100	
			3	14.6302°N, 80.0977°E	NLR-34449	60	20	628		157		25.00		100	
			4	14.6287°N, 80.0975°E	RNR-15048	65	20	538		9		1.67		30	
			5	14.6298°N, 80.0984°E	RNR-15048	65	20	582		11		1.89		40	
		Dandigunta	1	14.6184°N, 80.1016°E	RNR-15048	55	20	582	620	24	24.8	4.12	4.00	65	67
			2	14.6164°N, 80.1030°E	NLR-34449	60	20	635		26		4.09		70	
			3	14.6167°N, 80.1021°E	NDLR-7	50	20	594		22		3.70		60	
			4	14.6165°N, 80.1016°E	NDLR-7	65	20	663		28		4.22		65	
			5	14.6187°N, 80.1017°E	RNR-15048	55	20	626		24		3.83		75	
	Chittamur	**Buradagali**	1	13.9563°N, 80.0648°E	BPT-5204	63	20	391	334.2	31	42.2	7.90	12.94	30	79
			2	13.9556°N, 80.0649°E	BPT-5204	63	20	344		31		9.00		85	

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			3	13.9563°N, 80.0644°E	BPT-5204	63	20	290		52		17.90		95	
			4	13.9547°N, 80.0647°E	BPT-5204	63	20	327		56		17.10		95	
			5	13.9562°N, 80.0645°E	BPT-5204	63	20	319		41		12.80		90	
		Kothapalem	1	14.0689°N, 80.0488°E	RNR-15048	56	20	391	334.2	31	19.8	7.90	5.87	30	54
			2	14.0694°N, 80.0486°E	BPT-5204	65	20	344		12		3.49		50	
			3	14.0686°N, 80.0494°E	BPT-5204	65	20	290		17		5.86		60	
			4	14.0696°N, 80.0487°E	BPT-5204	65	20	327		20		6.12		60	
			5	14.0688°N, 80.0496°E	BPT-5204	65	20	319		19		5.96		70	
	Sullurupeta	**Illupuru**	1	13.7176°N, 79.9770°E	NDLR-7	68	20	359	322	23	20	6.41	6.51	80	53
			2	13.7183°N, 79.9770°E	BPT-5204	75	20	275		17		6.18		52	
			3	13.7167°N, 79.9779°E	BPT-5204	75	20	282		43		15.25		72	
			4	13.7197°N, 79.9768°E	RNR-15048	53	20	402		12		2.99		36	
			5	13.7180°N, 79.9740°E	BPT-5204	75	20	292		5		1.71		25	
		Vellukadu	1	13.7959°N, 80.0764°E	NLR-4001	90	20	808	803	442	369.2	54.70	45.30	100	100
			2	13.7951°N, 80.0772°E	NLR-4001	90	20	794		327		41.18		100	
			3	13.7965°N, 80.0770°E	NLR-4001	90	20	810		362		45.31		100	
			4	13.7975°N, 80.0785°E	NLR-4001	90	20	805		360		37.52		100	
			5	13.7978°N, 80.0767°E	NLR-4001	90	20	798		355		47.74		100	

Note: DAP: Days After Transplantation, TP: Total Plants, PD: Plant Damage, TT: Total Tillers, SS: Silver Shoots.

Table 4.2 Incidence of rice gall midge, *Orseolia oryzae* on rice in different rice growing mandals Southern zone of Andhra Pradesh during *kharif/ rabi* 2022.

S.No	District	Mandal	SS Mean (%)	PD Mean (%)
1	Chittoor	Sri Kalahasti	6.88	57.75
		Yerpedu	3.56	47.00
		Tirupati Rural	3.06	45.00
2	Y.S.R	Kadapa Rural	10.02	64.00
		Vallur	14.35	76.50
		Khajipet	12.22	87.50
3	Nellore	Vidavalur	10.83	70.50
		Chittamur	9.41	66.50
		Sullurupeta	25.91	76.50

Table 4.3 Incidence of rice gall midge, *Orseolia oryzae* on rice in different rice growing districts of Andhra Pradesh during *kharif/ rabi* 2022.

S.No	District	SS Mean (%)	PD Mean (%)
1	Chittoor	4.50	49.92
2	Y.S.R	12.19	76.00
3	Nellore	15.38	71.20

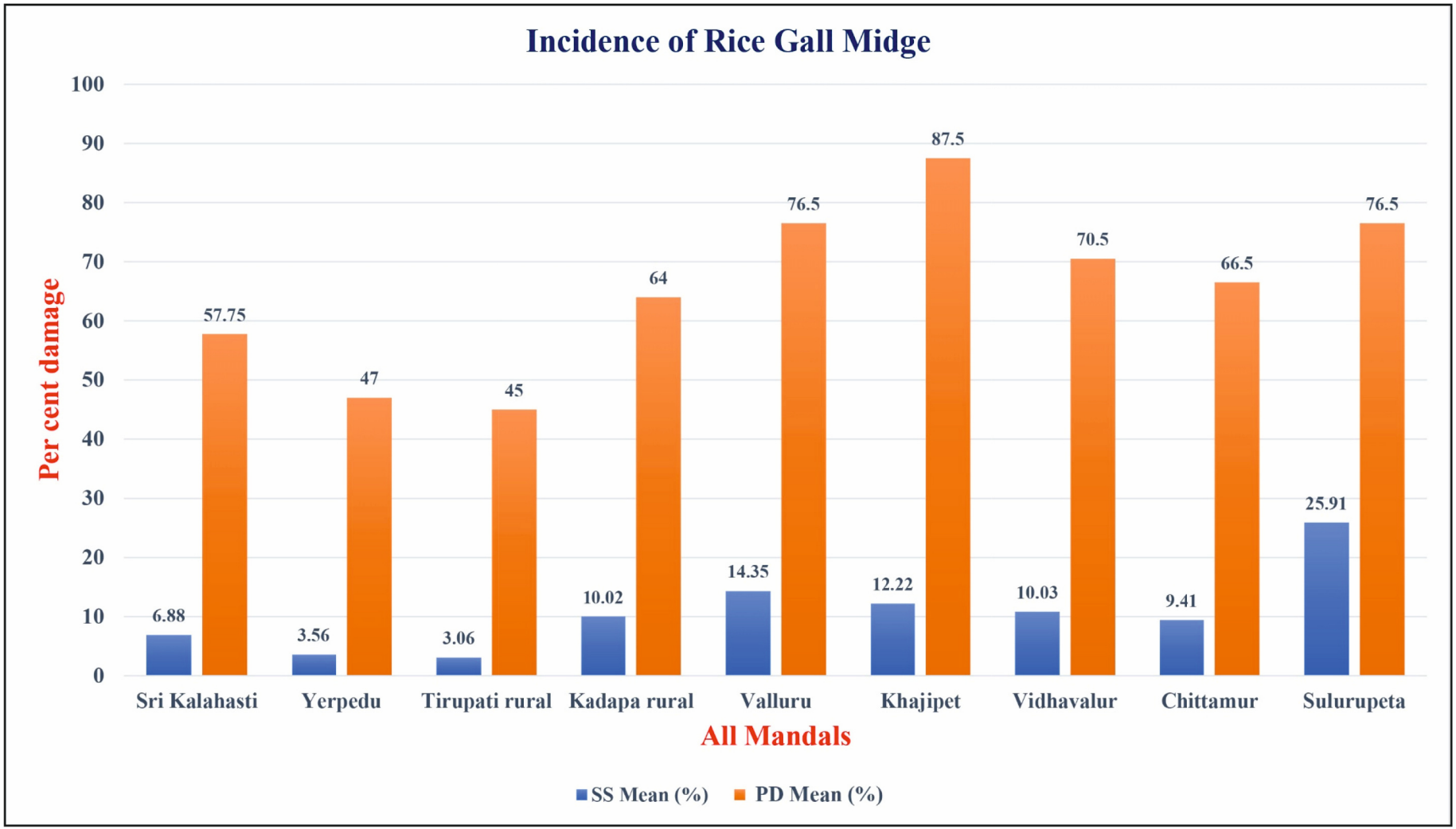


Fig. 4.1 Frequency for incidence of rice gall midge, *Orseolia oryzae* on rice in different rice growing mandals of Andhra Pradesh during *kharif/ rabi* 2022.

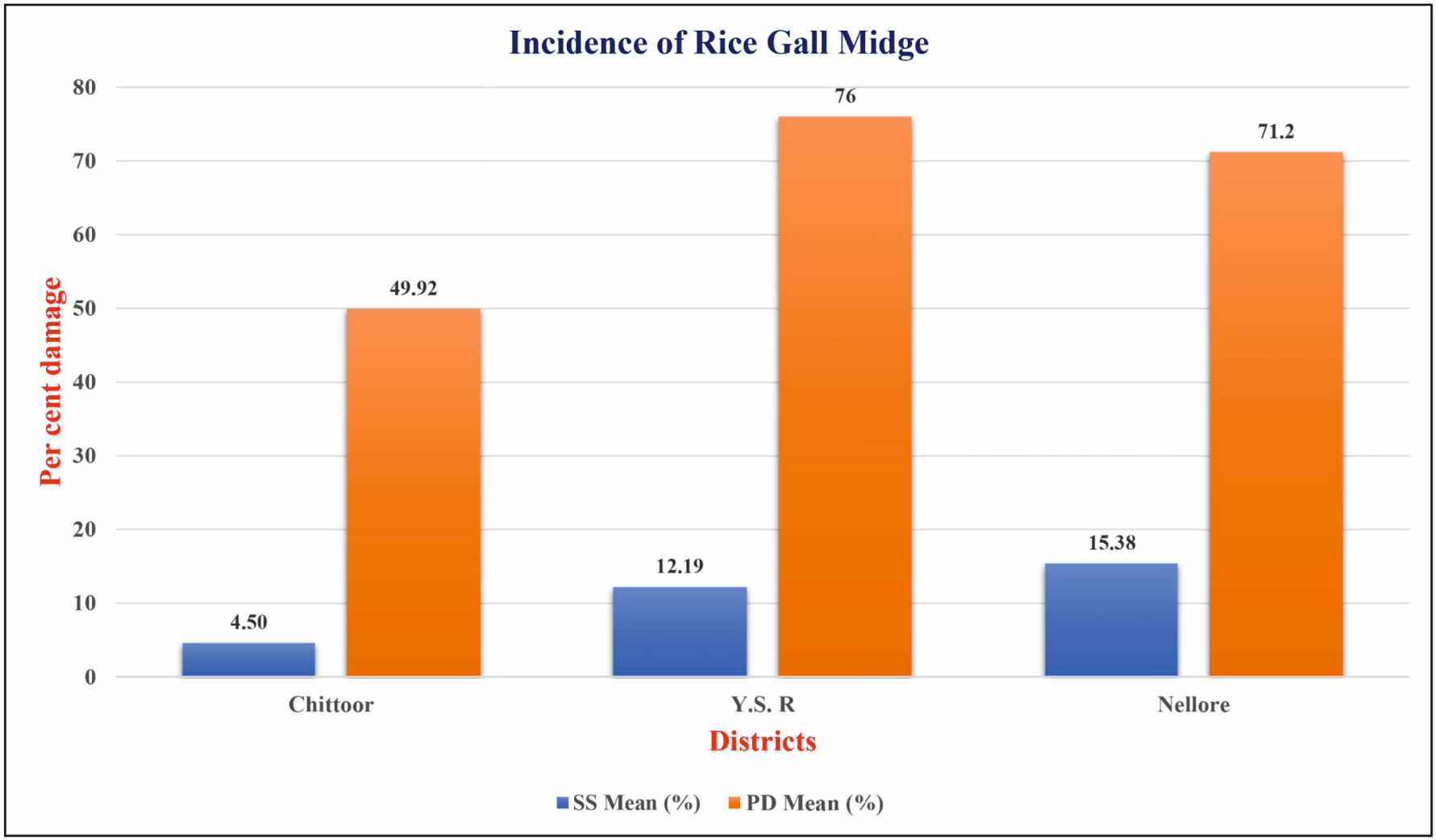


Fig. 4.2 Frequency for incidence of rice gall midge, *Orseolia oryzae* on rice in different rice growing districts Southern zone of Andhra Pradesh during *kharif/ rabi* 2022.

Surendra and Bindhu (2020) conducted roving survey on major insect pests of rice crop and recorded the silver shoot damage of 10.71 to 15.38 per cent in different blocks of Patna District of Bihar state during *kharif*, 2014-15.

4.2 DETECTION OF RICE GALL MIDGE BIOTYPE UNDER FIELD CONDITIONS IN Y.S.R DISTRICT AND ITS MOLECULAR IDENTIFICATION

4.2.1 Detection of Rice Gall Midge Biotype under field conditions

A set of 17 standard differentials representing five groups identified to characterize the prevailing gall midge biotypes in the country provided by Agricultural Research Station (ARS), Ragolu and 16 differentials provided by Indian Institute of Rice Research (IIRR), Hyderabad was sown and transplanted at ARS, Utukur, Kadapa, during *kharif*, 2022-23 to identify the existing biotype at Y.S.R District.

In first date of transplantation, the pest incidence at ARS, Utukur, Kadapa was low to severe. Per cent mean damage of silver shoot ranged from 0.00 to 3.03 percent at 30 DAT and 0.00 to 4.71 per cent at 50 DAT. Per cent mean plant damage ranged from 0.00 to 12.50 per cent at 30 DAT and 0.00 to 22.50 per cent at 50 DAT in both DAT's highest per cent was recorded in ABHAYA entry. In the case of susceptible check variety TN 1, the per cent mean silver shoot of 4.69 and 7.90 per cent at 30 and 50 DAT, while the per cent mean plant damage of 12.50 and 22.50 per cent was recorded at 30 DAT and 50 DAT respectively. (Table 4.4).

In Group I, apart from the entry KAVYA which exhibited susceptibility (S) at 50 DAT with silver shoot damage (2.98%) and plant damage (7.50%), all other entries shown resistance (R) with 0.00 per cent occurrence. In Group II, all entries showed susceptibility (S) except ARC 5984, which displayed resistance with 0.00 per cent of gall midge incidence at 30 and 50 DAT. Group III experienced susceptibility (S), with MR 1523 being the notable entry. In Group IV, INRC 3021 and AGANNI exhibited resistance (R) with 0.00 per cent occurrence of gall midge incidence. Finally, in Group V, TN 1, which was used as a susceptible check, showed susceptibility (S) as expected.

Among the 17 entries, a total of five entries, namely W 1263, ARC 6605, ARC 5984, INRC 3021, and AGANNI were found to be resistant with no incidence.

This study demonstrates that the observed reaction pattern closely aligns with biotype VI, which can be represented as R-S-S-S-S. This finding is consistent with the reaction pattern reported in Nellore District by (Harathi, 2019), providing additional support for the results.

In the second date of transplantation, the pest incidence ranged from low to very severe. Among the entries, PHALGUNA and RP-2333-156-8 exhibited similar levels of damage as the susceptible check at 50 DAT. At 30 DAT, the per cent mean of silver shoot damage varied from 0.00 to 15.81 per cent, while at 50 DAT, it ranged from 0.00 to 29.63 per cent. The recorded per cent of plant damage at 30 DAT and 50 DAT ranged from 0.00 to 77.50 per cent and 0.00 to 100.00 per cent, respectively. In the case of the susceptible check variety TN 1, the per cent of silver shoot damage at 30 DAT and 50 DAT was 20.78 and 35.82 per cent respectively. Regarding plant damage, TN 1 exhibited 82.50 and 100.00 per cent at 30 DAT and 50 DAT, respectively (Table 4.5).

In Group I, all entries except for KAVYA showed resistance (R), with a no occurrence of gall midge incidence. However, KAVYA exhibited susceptibility (S) at 50 DAT with 4.63 per cent silver shoot damage and 15.50 per cent plant damage. In Group II, ARC 5984 was the only entry that demonstrated resistance (R), with no gall midge incidence at both 30 and 50 DAT. All other entries in this group were susceptible (S) to the pest. Group III had MR 1523 as the notable entry, showing susceptibility (S) to the gall midge. In Group IV, both INRC 3021 and AGANNI displayed resistance (R), with nil of gall midge incidence. As for group V, TN 1, which served as the susceptible check, exhibited the expected susceptibility (S) to the pest.

Among the 17 entries, a total of five entries, namely W 1263, ARC 6605, ARC 5984, INRC 3021, and AGANNI, were found to be resistant (R) with no incidence of gall midge.

In case of field studies on 16 differentials obtained from IIRR, Hyderabad, no damage was observed in the entries W 1263, ARC 6605, ARC 5984, INRC 3021, and AGANNI, despite the pest incidence ranging from moderate to severe. The per cent mean silver shoot damage varied from 0.00 to 15.07 per cent at 30 DAT and 0.00 to 23.84 per cent at 50 DAT. In terms of per cent mean plant damage, it ranged from 0.00 to 52.50 per cent at 30 DAT and 0.00 to 85.00 per cent at 50 DAT. Comparatively, the susceptible check variety TN 1 recorded 21.01 and 30.86 per cent silver shoot damage at 30 DAT and 50 DAT, respectively. TN 1 also exhibited 50.00 and 95.00 per cent plant damage at 30 DAT and 50 DAT, respectively (Table 4.6).

In Group I, all entries, except for KAVYA, had shown resistance (R) with nil incidence of gall midge at 30 DAT. However, KAVYA was susceptible (S) at 50 DAT, showing 7.32 per cent silver shoot damage and 24.50 per cent plant damage. Within Group II, ARC 5984 was the sole entry that exhibited resistance (R), with no gall midge incidence at both 30 and 50 DAT. All other entries in this group were susceptible (S) to the pest. Group III featured MR 1523 as the notable entry, displaying susceptibility (S) to the gall midge. In Group IV, both INRC 3021 and AGANNI showcased resistance (R) with no occurrence of gall midge incidence. Lastly, in Group V, TN 1, serving as the susceptible check, exhibited the expected susceptibility (S) to the pest.

Based on the results obtained, it is clear that the gall midge biotype present at ARS, Utukur, Kadapa, Y.S.R District does not exhibit any specific resistant or susceptible reaction to the studied group of differentials. Furthermore, it does not follow any known or identified biotype pattern. Therefore, further investigations are required to determine the specific biotype present in Kadapa. The recorded data does not align with the prescribed biotype pattern for the following reasons:

a. During the first transplantation, the pest incidence was very low, and the damage observed in the susceptible check was below the threshold of 60 per cent. According to the experimental criteria, the recorded data is considered valid only when the plant damage in the susceptible check exceed 60 per cent.

b. The pest did not demonstrate a consistent biotype reaction pattern among the differentials tested. In Group I, the KAVYA entry exhibited a different reaction compared to the other entries in the same group. Similarly, in Group II, the entry ARC 5984, and in Group IV, the entries INRC 3021 and AGANNI displayed distinct reactions compared to the remaining entries in their respective groups.

Therefore, additional investigations and analysis are necessary to identify and understand the specific biotype existing in Kadapa, as the recorded data deviates from the expected biotype pattern.

To assess the similarity of resistance or susceptibility reactions of each differential within the prescribed biotype patterns, per cent similarity index (PSI) was calculated for the particular biotype. The results revealed that the unknown biotype at Kadapa shares a 76.47% similarity with biotype VI, followed by biotype IVM at 64.7%. Biotype III and IV exhibited a similarity index of 58.82%, while biotype V showed a similarity of 47.05%. Biotype I and II had similarity indices of 35.29% and 29.41%, respectively (Table 4.7). Based on these similarity index values, the biotype present in Utukur, Kadapa can be designated as biotype VI. The per cent similarity index parameter is a helpful tool in determining the degree of similarity between resistance or susceptibility reactions of differentials and gall midge populations in cases where confirming the biotype through conventional differential patterns is challenging. Further investigations have to be carried out to confirm the existence of gall midge biotype VI in Kadapa.

Table 4.4 Reaction of differentials (ARS, Ragolu) to the existing biotype at ARS, Utukur during late *kharif* in first date of transplantation (08-08-2022).

Group	Entry No.	Differential	Silver shoot damage (%)		Plant damage (%)		Reaction observed
			30 DAT	50 DAT	30 DAT	50 DAT	
I	1	KAVYA	0.00 (0.00)	2.98 (9.93)	0.00 (0.00)	7.50 (15.89)	S
	2	W 1263	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	3	ARC 6605	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
II	4	PHALGUNA	0.44 (3.80)	2.84 (9.69)	5.00 (12.92)	10.00 (36.87)	S
	5	ARC 5984	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	6	DUKONG - 1	1.36 (6.70)	4.29 (11.95)	5.00 (12.92)	12.50 (41.22)	S
	7	RP-2333-156-8	1.11 (6.05)	3.70 (11.09)	5.00 (12.92)	12.50 (41.22)	S
	8	MADHURI L 9	0.32 (3.19)	2.40 (8.90)	5.00 (12.92)	10.00 (36.87)	S
	9	BG-380-2	0.81 (5.16)	2.99 (9.96)	5.00 (12.92)	15.00 (45.57)	S
III	10	MR 1523	0.23 (2.72)	2.30 (8.72)	5.00 (12.92)	10.00 (36.87)	S
IV	11	RP 2068-18-3-5	0.42 (3.72)	2.83 (9.68)	7.50 (15.68)	12.50 (41.22)	S
	12	ABHAYA	3.03 (10.00)	4.71 (12.53)	12.50 (20.70)	22.50 (56.57)	S
	13	INRC 3021	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	14	AGANNI	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	15	INRC 15888	1.41 (6.81)	4.46 (12.18)	10.00 (18.43)	15.00 (45.57)	S
	16	B 95-1	0.23 (1.92)	2.09 (8.31)	2.50 (9.10)	10.00 (36.87)	S
V	17	TN 1	4.69 (12.51)	7.90 (16.32)	12.50 (20.70)	22.50 (56.57)	S
		Grand Mean	0.83	2.56	4.41	9.41	
		F Value	4.42	4.05	4.57	4.02	
		P Value (0.05)	0.001	0.001	0.001	0.001	
		Sig	S	S	S	S	

Note: Values in the parenthesis are Angular transformation; DAT: Days After Transplanting

Table 4.5 Reaction of differentials (ARS, Ragolu) to the existing biotype at ARS, Utukur during late *kharif* in second date transplantation (23-09-2022).

Group	Entry No.	Differential	Silver shoot damage (%)		Plant damage (%)		Reaction observed
			30 DAT	50 DAT	30 DAT	50 DAT	
I	1	KAVYA	0.00 (0.00)	4.63 (12.43)	0.00 (0.00)	15.50 (23.18)	S
	2	W 1263	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	3	ARC 6605	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
II	4	PHALGUNA	11.43 (19.75)	29.63 (32.98)	55.00 (47.87)	100.00 (90.00)	S
	5	ARC 5984	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	6	DUKONG - 1	10.91 (19.28)	15.43 (23.12)	30.00 (33.21)	82.50 (65.27)	S
	7	RP-2333-156-8	11.87 (20.14)	24.38 (29.59)	77.50 (61.68)	97.50 (80.90)	S
	8	MADHURI L 9	10.98 (19.28)	21.96 (27.94)	25.00 (30.00)	67.50 (55.25)	S
	9	BG-380-2	14.15 (22.10)	20.28 (26.75)	52.50 (46.43)	80.00 (63.43)	S
III	10	MR 1523	12.96 (21.10)	27.36 (31.54)	42.50 (40.69)	82.50 (65.27)	S
IV	11	RP 2068-18-3-5	10.90 (19.28)	24.43 (29.61)	35.00 (36.27)	77.50 (61.68)	S
	12	ABHAYA	15.81 (23.42)	24.15 (29.43)	52.50 (46.43)	82.50 (65.32)	S
	13	INRC 3021	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	14	AGANNI	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	15	INRC 15888	4.49 (12.23)	9.09 (17.54)	22.50 (28.32)	52.50 (46.43)	S
	16	B 95-1	14.66 (22.51)	18.53 (25.49)	32.50 (34.76)	65.00 (53.78)	S
V	17	TN 1	20.78 (27.12)	35.82 (36.76)	82.50 (65.27)	100.00 (90.00)	S
		Grand Mean	8.17	15.04	29.85	53.12	
		F Value	4.00	4.02	3.99	4.32	
		P Value (0.05)	0.001	0.001	0.001	0.001	
		Sig	S	S	S	S	

Note: Values in the parenthesis are Angular transformed; DAT: Days After Transplanting

Table 4.6 Reaction of differentials (IIRR, Hyderabad) to the existing biotype at ARS, Utukur during late *kharif* on date transplantation (13-10-2022).

Group	Entry No.	Differential	Silver shoot damage (%)		Plant damage (%)		Reaction observed
			30 DAT	50 DAT	30 DAT	50 DAT	
I	1	KAVYA	0.00 (0.00)	7.32 (15.70)	0.00 (0.00)	24.50 (29.67)	S
	2	W 1263	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	3	ARC 6605	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
II	4	PHALGUNA	0.68 (4.73)	4.26 (11.90)	5.00 (12.92)	27.50 (31.63)	S
	5	ARC 5984	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	6	DUKONG - 1	5.98 (14.15)	23.34 (28.89)	30.00 (33.21)	77.50 (61.68)	S
	7	RP-2333-156-8	11.55 (19.86)	10.00 (18.43)	10.00 (18.43)	80.00 (63.43)	S
	8	MADHURI L 9	9.31 (17.76)	17.49 (24.72)	45.00 (42.13)	72.50 (58.37)	S
	9	BG-380-2	9.23 (17.79)	19.94 (26.52)	32.50 (34.76)	77.50 (61.68)	S
III	10	MR 1523	12.88 (21.03)	18.14 (25.21)	52.50 (46.43)	75.00 (60.00)	S
IV	11	RP 2068-18-3-5	15.07 (22.84)	20.53 (26.94)	47.50 (43.57)	72.50 (58.37)	S
	12	ABHAYA	2.28 (8.68)	19.98 (26.55)	15.00 (22.79)	77.50 (61.68)	S
	13	INRC 3021	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	14	AGANNI	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	R
	15	INRC 15888	2.14 (8.41)	23.84 (29.23)	10.00 (18.43)	85.00 (67.21)	S
V	16	TN 1	21.01 (27.28)	30.86 (33.74)	50.00 (45.00)	95.00 (77.08)	S
		Grand Mean	5.63	12.23	18.59	47.78	
		F Value	4.01	4.02	4.10	4.02	
		P Value (0.05)	0.001	0.001	0.001	0.001	
		Sig	S	S	S	S	

Note: Values in the parenthesis are Angular transformed; DAT: Days After Transplanting

From the data provided in tables 4.4, 4.5, and 4.6, it is clear that a significant number of entries in Group I exhibited resistance to the unknown biotype of rice gall midge population present in Utukur, Kadapa.

Based on the 2017 annual report of IIRR, the biotype observed at their Hyderabad location displayed a distinct pattern of resistance-resistance-resistance-resistance-susceptibility (R-R-R-R-S). Interestingly, the populations at Jagdalpur also followed the same pattern, while at IIRR, the Duokang-1 and BG308-2 populations exhibited susceptibility. On the other hand, at Jagtial and Ranchi, the populations displayed the typical resistance-susceptibility-resistance-resistance-susceptibility (R-S-R-R-S) pattern for biotype 3, except for RP 2068-18-3-5, which showed susceptibility at both locations.

In a study conducted by Harathi (2019) at Nellore, a different reaction pattern was observed. To determine the biotype, present at Nellore, they employed the Per cent Similarity Index (PSI) and found that it exhibited a 70.6% similarity with biotype VI. This finding suggests that the biotype observed at Nellore shares a significant similarity with biotype VI, as determined by the PSI calculation.

According to a study conducted by Sunitha *et. al*. (2021) at RARS, Warangal, the recorded reaction pattern of gall midge did not align with any of the seven prescribed biotypes. This suggests a change in the reaction pattern of the standard differentials towards biotype 4M, which exhibited virulence against the resistant group IV rice differentials. This indicates a potential shift or emergence of a new biotype in the region.

In contrast, Lingaraj *et. al.* (2008) reported a different finding in Madikeri and Ponnampet, Kodagu, Mysore, and Hassan districts of Karnataka. In his study, he observed a perfect reaction pattern with the presence of biotype I, which showed an R-R-R-S reaction pattern when tested against 14 rice differentials. These findings demonstrate regional variations in the gall midge biotype distribution and the associated reaction patterns.

In the study conducted by Vijay Kumar *et. al.* (2022) employed three differentials, namely W 1263, PHAGUNA, and TN-1. The researchers observed a progressive change in the virulence spectrum of the local gall midge biotype II,

which was capable of causing damage to resistant varieties in the south coast region. Additionally, they discovered the presence of a new biotype, biotype III, for the first time in the north coast of Karnataka. This new biotype exhibited virulence characteristics that were distinct from the previously identified biotypes. These findings highlight the dynamic nature of gall midge populations and the evolution of new biotypes with different virulence patterns in different regions.

Paramasiva *et. al.* (2023) investigated the changing reaction pattern of rice gall midge at the Agricultural Research Station in Nellore during *kharif* seasons from 2015-16 to 2021-22. Using 17 standard differentials from IIRR, Hyderabad, they observed a dynamic shift in resistance and susceptibility. Entries AGANNI and INRC 15888 showed resistance in 2015 and 2019, but all three entries AGANNI, INRC 15888, and INRC 3021 displayed susceptibility in 2021. The study also revealed a high similarity index with biotype VI, indicating a characteristic response pattern.

Indeed, continuous biotype monitoring studies are crucial in various aspects of rice gall midge management. These studies provide valuable insights into the biotype dynamics and aid in identifying resistant donors for the development of resistant cultivars. By monitoring the biotype over time, researchers can track any changes or shifts in the population, allowing for proactive measures to be taken.

Biotype monitoring studies are particularly useful for identifying and selecting resistant genes, which can be incorporated into new cultivars through breeding programs. This process may involve pyramiding or stacking multiple resistant genes to enhance the durability of resistance against the gall midge. By monitoring the biotype, breeders can assess the effectiveness of these resistance genes and make informed decisions regarding their deployment.

Table 4.7 Per cent Similarity Index of existing biotype at Kadapa in comparison with all the seven biotypes existing in India

Group	Entry No.	Differential	Resistant gene	Reaction at Kadapa	Gall midge Biotype (Prescribed Reaction Pattern)						
					1	2	3	4	4M	5	6
I	1	KAVYA	*Gm1*	S	R	S	R	S	S	R	R
	2	W 1263	*Gm1*	R	R	S	R	S	S	R	R
	3	ARC 6605		R	R	S	R	S	S	R	R
II	4	PHALGUNA	*Gm2*	S	R	R	S	S	S	R	S
	5	ARC 5984	*Gm5*	R	R	R	S	S	S	R	S
	6	DUKONG - 1	*Gm6*	S	R	R	S	S	S	R	S
	7	RP-2333-156-8	*Gm7*	S	R	R	S	S	S	R	S
	8	MADHURI L 9	*Gm9*	S	R	R	S	S	S	R	S
	9	BG-380-2	*Gm10*	S	R	R	S	S	S	R	S
III	10	MR 1523	*Gm11*	S	R	R	R	R	S	R	S
IV	11	RP 2068-18-3-5	*gm3*	S	R	R	R	R	S	S	S
	12	ABHAYA	*Gm4*	S	R	R	R	R	R	S	S
	13	INRC 3021		R	R	R	R	R	R	S	S
	14	AGANNI	*Gm8*	R	R	R	R	R	R	S	S
	15	INRC 15888		S	R	R	R	R	R	S	S
	16	B 95-1		S	R	R	R	R	R	S	S
V	17	TN 1	None	S	S	S	S	S	S	S	S
	Percent Similarity Index				**35.29**	**29.41**	**58.82**	**52.94**	**64.70**	**47.05**	**76.47**

4.2.2 Molecular Identification of Rice Gall Midge Biotype

Rice gall midge (*Orseolia oryzae*) constitutes one of the major insect pests in paddy. *O. oryzae* complex consists of seven biotypes in India which are indistinguishable and difficult to classify them into biotypes using morphological characters. Recently rapid molecular identification of insect species has become popular and can be used as an alternative to traditional taxonomic methods. DNA-based molecular markers are used for identification of species and their use represents a valuable addition or alternative to traditional taxonomic identification methods. Phylogenetic analysis is viewed as a valuable tool for pest management. Hence, we attempted to identify the genetic groups/ biotype of *O. oryzae* population from major rice growing districts of Southern zone of Andhra Pradesh.

4.2.2.1 Molecular identification of *O. oryzae* biotype in Southern zone of Andhra Pradesh.

The genomic DNA was isolated from the gall midge population collected in three districts of Southern zone of Andhra Pradesh *viz*., Chittoor, Y.S.R and Nellore districts. From three districts, five samples were collected such as Chittoor I (Vedam), Chittoor II (Peruru), Kadapa (Utukur), Nellore I (Vellukadu) and Nellore II (Gadeladhinne). The isolated genomic DNA from single adult female gall midge collected from each sample was extracted and amplified with specific set of primers targeting such as partial mitochondrial Cytochrome Oxidase -I (mtCOI), Simple Sequence Repeats (SSR's) and Sequence Characterized Amplified Regions (SCAR's). A total of six SSR markers, (Oosat16, Oosat21, Oosat24, Oosat35, Oosat43, and Oosat59) along with two SCAR markers (Y132 and Y133) and one mtCOI marker, were employed to amplify the gall midge samples collected from various locations of Southern zone of Andhra Pradesh.

4.2.2.2 Molecular identification of *O. oryzae* biotype by using SSR's primers (Simple Sequence Repeats)

SSR primers were utilized to identify three distinct biotypes: GMB1, GMB4, and GMB4M, from a set of five samples. Identification was done based on the band fragment size observed for each biotype-specific primer. For the SSR primers Oosat21 (150-160 bp), Oosat35 (180-200 bp), and Oosat43 (180-200 bp),

the range of band fragment sizes in the five samples aligned with the range provided by Bentur *et. al*. (2011). This suggests that the primers Oosat21, Oosat35, and Oosat43 may correspond to biotypes 1, 4, and 4M, respectively. However, it should be noted that the Kadapa (Utukur) sample did not produce an amplified band fragment with the Oosat35 primer. On the other hand, the primers Oosat16, Oosat24, and Oosat59, amplified at the band fragment sizes (260-280 bp, 350-400 bp, and 120-160 bp) differed significantly from the expected band fragment sizes of GMB1, GMB4, and GMB4M (153-162 bp, 160-200 bp and 78-107 bp) (Table 4.8 and Plate 4.1 to 4.3). This study revealed that mixed population may be prevailing in the Southern zone of Andhra Pradesh, Chittoor I (Vedam), Chittoor II (Peruru), Kadapa (Utukur), Nellore I (Vellukadu), and Nellore II (Gadeladhinne).

4.2.2.3 Molecular identification of *O. oryzae* biotype by using SCAR's (Sequence Characterized Amplified Regions)

According to Behura *et. al*. (1999), the SCAR markers Y132 and Y133 exhibited specificity for biotype 5 and biotype 4 respectively, with band fragment sizes of approximately 600 bp and 750 bp. Using SCAR marker Y132, the DNA samples of insects collected from Nellore I (Vellukadu), Nellore II (Gadeladhinne), Chittoor I (Vedam), and Chittoor II (Peruru) were amplified at an annealing temperature of 61°C but the Kadapa (Utukur) did not undergo amplification. The resulting fragment lengths exactly matched the band fragment sizes (600 bp) reported by Behura *et. al*. (1999), providing clear evidence that confirm the samples except Kadapa (Utukur) sample belong to biotype 5 (Table 4.9 and Plate 4.4) and Kadapa (Utukur) sample may belong to other biotype.

Using the Y133 SCAR marker, all five DNA samples Kadapa (Utukur), Nellore I (Vellukadu), Nellore II (Gadeladhinne), Chittoor I (Vedam), and Chittoor II (Peruru) were subjected to amplification at an annealing temperature of 55°C. However, the observed band fragment size in all five samples was 1000 bp, which differed significantly from the expected band fragment size of 750 bp reported by Behura *et. al*. (1999) for biotype 4 (Table 4.9 Plate 4.4). This

discrepancy indicates that none of the five samples belong to biotype 4 and suggests the possibility of other biotypes in Southern zone of Andhra Pradesh.

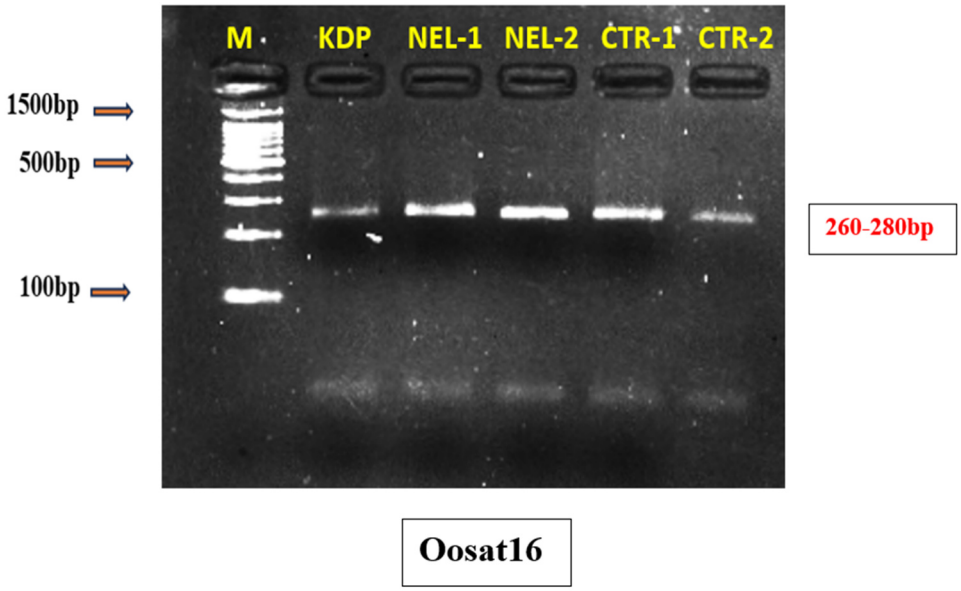
Based on the aforementioned information, the SSR primers Oosat21, Oosat35, and Oosat43 were found to correspond to biotypes 1, 4, and 4M respectively. Conversely, the observed range of band fragment sizes for the SSR primers Oosat16, Oosat24, and Oosat59 showed significant variations, indicating differences in the targeted biotypes. With the SCAR marker Y132, four samples clearly exhibited the presence of biotype 5 except Kadapa (Utukur), as Y132 is specific to biotype 5 with a band fragment size of 600 bp. However, the other SCAR marker, Y133, yielded band fragments (1000 bp) different from the expected band fragment size (750 bp) for biotype 4 in all five samples.

Subsequently, the study progressed to identify the biotypes found in the sampled locations within the Southern zone of Andhra Pradesh by implementing additional procedures. The mitochondrial genome is particularly notable due to its characteristics of maternal inheritance, highly conserved, lack of recombination, and the existence of sequence polymorphisms among diverse taxa. The rearrangement of genes within mitochondrial genomes is highly informative for evolutionary investigations. In this regard, the repetitive sequences of mtCOI served as a valuable tool not only for distinguishing different gall midge biotypes but also for distinguishing *Orseolia* species more precise as demonstrated by Atray *et. al*. (2015).

4.2.2.4 Molecular characterization of *O. oryzae* biotype using partial mitochondrial CO-I primers

The extracted DNA from five samples of *O. oryzae* biotype were amplified with partial mitochondrial Cytochrome Oxidase-I primer and the PCR product was obtained at 600 bp (Plate 4.5, Table 4.10). The amplified PCR product was sent for sequencing. The PCR product was purified and sequenced at Eurofin Genomics India Pvt. Ltd., Bengaluru (Appendix III) and five sample populations were sequenced and submitted to NCBI GenBank and accession numbers (Table 4.12)

Five sample populations of the gall midge, along with seven known biotypes of *O. oryzae* from India, were subjected to nucleotide sequencing.



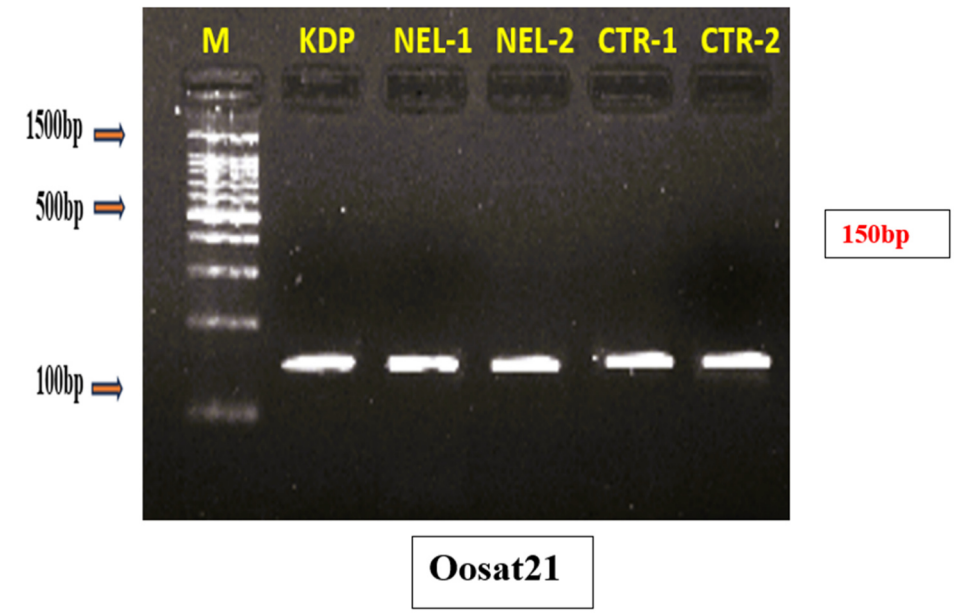


Plate 4.1: Genetic variability of rice gall midge samples across Southern zone of Andhra Pradesh using SSR's (Oosat16 and Oosat21), M: Marker(100bp), KDP- Kadapa, NEL-1: Nellore-1, NEL-2: Nellore-2, CTR-1: Chittoor-1 and CTR-2: Chittoor-2.

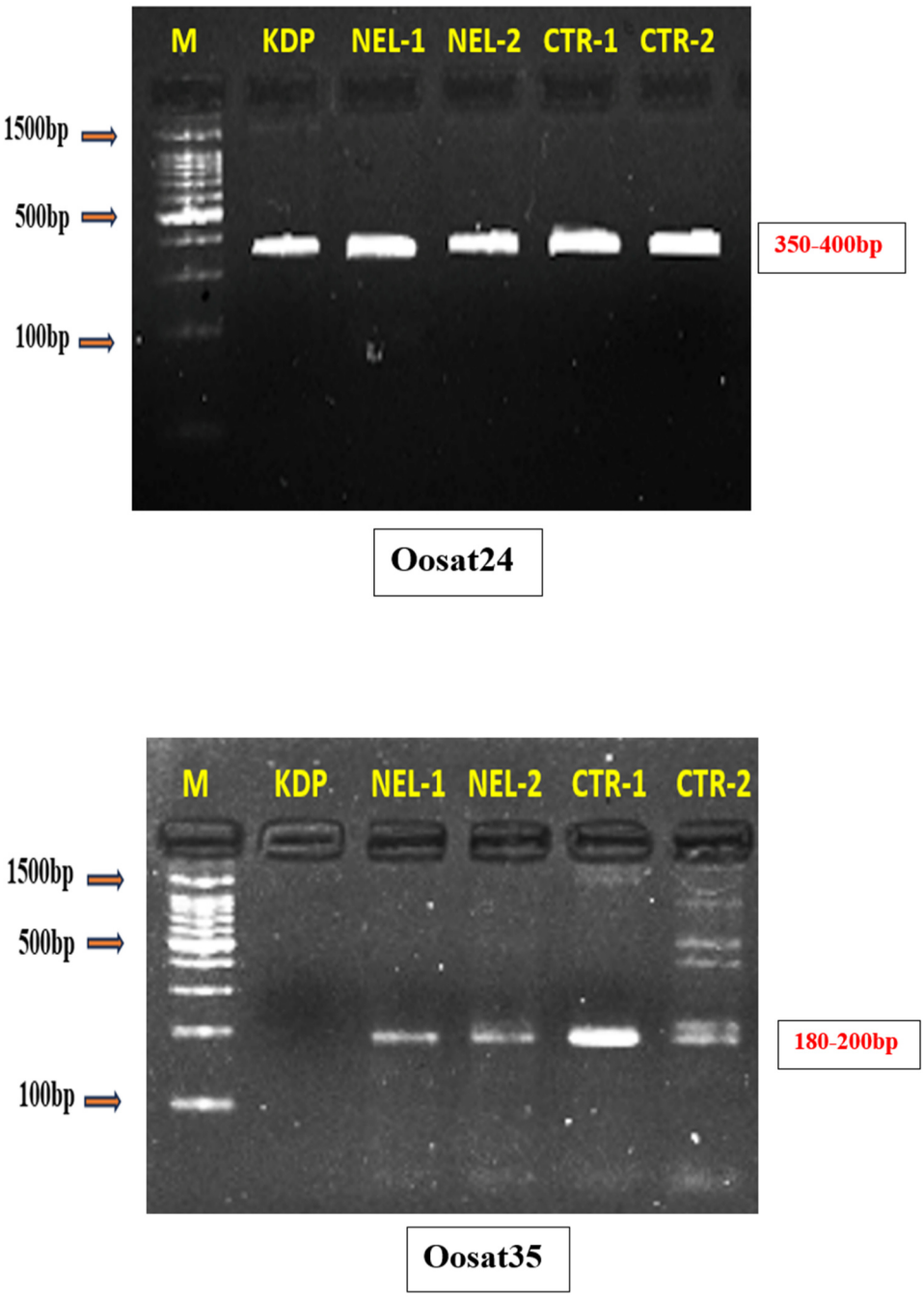


Plate 4.2: Genetic variability of rice gall midge samples across Southern zone of Andhra Pradesh using SSR's (Oosat24 and Oosat35), M: Marker(100bp), KDP- Kadapa, NEL-1: Nellore-1, NEL-2: Nellore-2, CTR-1: Chittoor-1 and CTR-2: Chittoor-2.

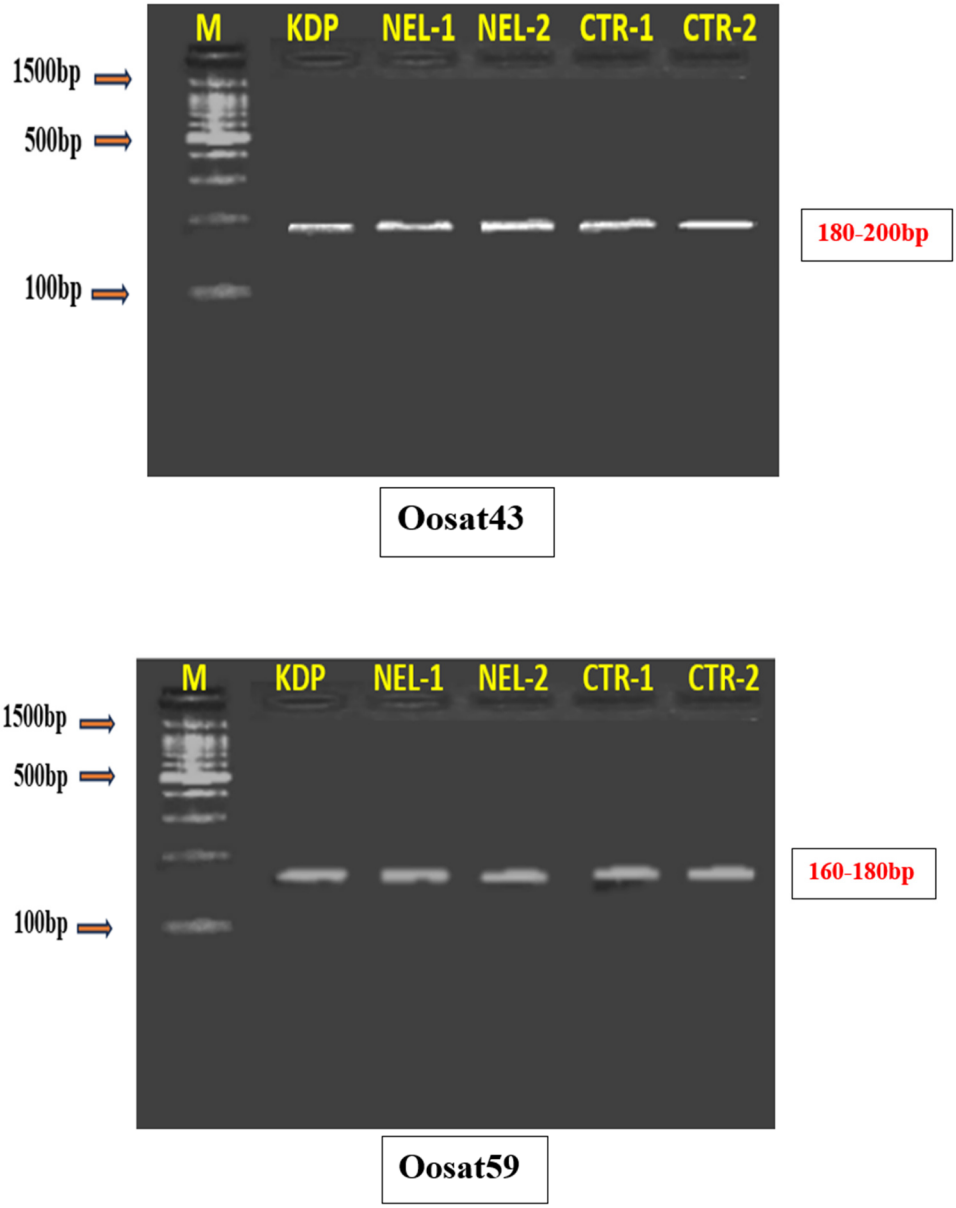
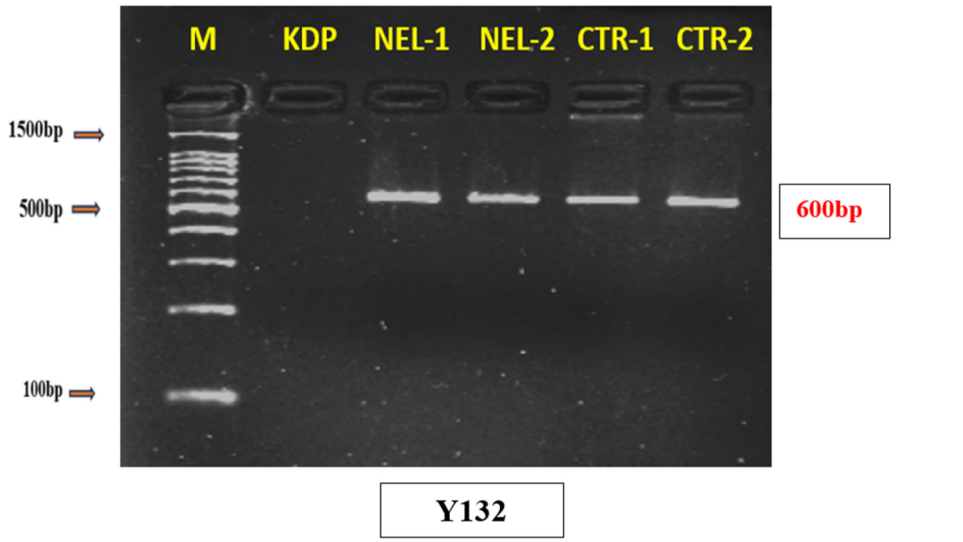


Plate 4.3: Genetic variability of rice gall midge samples across Southern zone of Andhra Pradesh using SSR's (Oosat43 and Oosat59), M: Marker(100bp), KDP-Kadapa, NEL-1: Nellore-1, NEL-2: Nellore-2, CTR-1: Chittoor-1 and CTR-2: Chittoor-2.



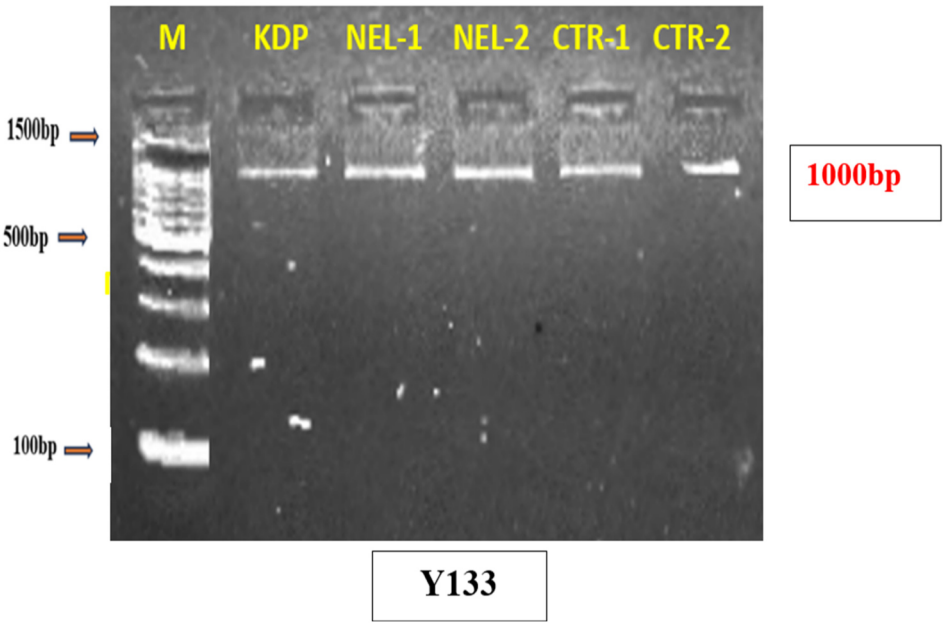


Plate 4.4: Genetic variability of rice gall midge samples across Southern zone of Andhra Pradesh using SCAR's (Y132 and Y133), M: Marker(100bp), KDP- Kadapa, NEL-1: Nellore-1, NEL-2: Nellore-2, CTR-1: Chittoor-1 and CTR-2: Chittoor-2.

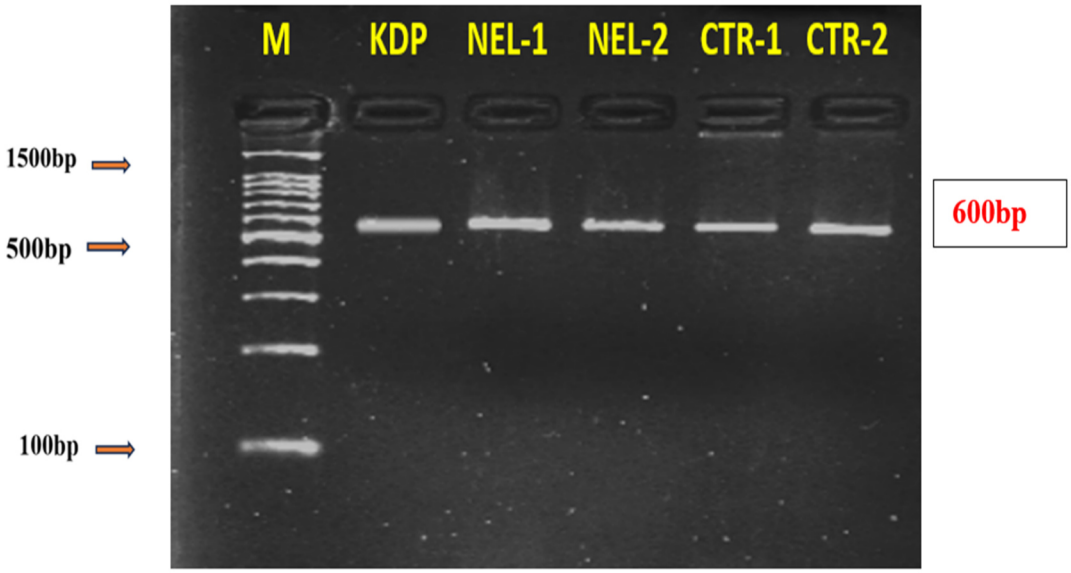


Plate 4.5: Genetic variability of rice gall midge samples across Southern zone of Andhra Pradesh using mtCOI, M: Marker(100bp), KDP- Kadapa, NEL-1: Nellore-1, NEL-2: Nellore-2, CTR-1: Chittoor-1 and CTR-2: Chittoor-2.

Table 4.8 List of SSR's primers/ makers, range of band size for distinguish GMB1, 4 and 4M biotypes in five sample populations.

S. No	SSR Primer/ Marker	Annealing temperature	Range of band size for biotypes GMB1, 4 and 4M	Range of band size with five samples of study
1	Oosat16	58 ^{o}C	153-162bp	260-280bp
2	Oosat21	55 ^{o}C	136-150bp	150-160bp
3	Oosat24	55 ^{o}C	160-200bp	350-400bp
4	Oosat35	55 ^{o}C	185-220bp	180-200bp
5	Oosat43	58.8 ^{o}C	165-188bp	180-200bp
6	Oosat59	55 ^{o}C	78-107bp	120-160bp

Table 4.9 List of SCAR's primers/ makers, range of band size for distinguish the biotypes in five sample populations.

S. No	SCAR Primer/ Marker	Annealing temperature	Range of band size	Range of band size with five samples of study
1	Y132	61 ^{o}C	600bp	600bp
2	Y133	55 ^{o}C	750bp	1000bp

Table 4.10 List of mtCOI primer range of band size for distinguish the biotype in five sample populations.

S. No	Primer	Annealing temperature	Range of band size	Range of band size with five samples of study
1	mtCOI	55^{o}C	600bp	600bp

Additionally, blasted sequences of other *O. oryzae* and one dipteran species, *Culex quinquefasciatus*, were included as outgroups. The sequences collected from the NCBI GenBank were also incorporated in the analysis. The alignment of these sequences was performed using Bio Edit 7 and MEGA 11 software, as described by Atray *et. al.* (2015) (Table 4.11) (Appendix-III).

4.2.2.5 Construction of Phylogenetic tree for Rice gall midge, *O. oryzae* of five sample populations of Southern zone of Andhra Pradesh.

Phylogenetic analysis using mtCO-I sequences of *Orseolia oryzae* population of three districts of Southern zone of Andhra Pradesh *viz.,* Chittoor, Y.S.R, Nellore district. From three districts, five samples were collected such as Chittoor I (Vedam), Chittoor II (Peruru), Kadapa (Utukur), Nellore I (Vellukadu) and Nellore II (Gadeladhinne) populations was carried out along with seven biotypes of *O. oryzae* of India and other *O. oryzae* (15 from Thailand and three from India) sequences were mined from NCBI GenBank by using Maximum Likelihood method (Fig. 4.3).

The constructed phylogenetic tree revealed two distinct clades. In one clade, biotypes of *O. oryzae* are GMB1, GMB2, GMB3, GMB4, GMB4M, GMB6 and Kadapa sample and in other clade includes GMB5 and the other samples Southern zone such as Nellore-I and II, Chittoor-I and II. The *O. oryzae* sample from Nellore-I (Vellukadu) and Nellore-II (Gadeladhinne) of Andhra Pradesh have shown close relationship and formed a separate cluster. The populations from Vellukadu and Gadeladhinne have shown close relationship and formed a cluster with each other.

The genetic groups Nellore-I (OR142658) and II (OR122465), Chittoor-I (OR143373) and II (OR241367) of present study shared a string bootstrap support of 96 per cent with *O. oryzae* population GMB5 (KP109817) of India.

Genetic group Utukur, Kadapa (OR141889) was shared a string bootstrap support of 98 per cent with *O. oryzae* population GMB6 (KP109818) of India.

Table 4.11 Mitochondrial partial genome CO I sequences of *O. oryzae* biotypes and other groups selected from NCBI for phylogenetic tree construction.

S.No	Species	Biotype	Accession Number
1	*Orseolia oryzae*	GMB1	KM888183
2	*Orseolia oryzae*	GMB2	KP261027
3	*Orseolia oryzae*	GMB3	KP261028
4	*Orseolia oryzae*	GMB4	KP109815
5	*Orseolia oryzae*	GMB4M	KP109816
6	*Orseolia oryzae*	GMB5	KP109817
7	*Orseolia oryzae*	GMB6	KP109818
8	*Orseolia oryzae*	-	OQ186757
9	*Orseolia oryzae*	-	KC506565
10	*Orseolia oryzae*	-	MT273882
11	*Orseolia oryzae*	-	MT273880
12	*Orseolia oryzae*	-	MT273883
13	*Orseolia oryzae*	-	MT273884
14	*Orseolia oryzae*	-	MT273886
15	*Orseolia oryzae*	-	MT273889
16	*Orseolia oryzae*	-	MT273890
17	*Orseolia oryzae*	-	MT273891
18	*Orseolia oryzae*	-	KX447436
19	*Orseolia oryzae*	-	KX447441
20	*Orseolia oryzae*	-	KX447439
21	*Orseolia oryzae*	-	KX447433
22	*Orseolia oryzae*	-	KX447442
23	*Orseolia oryzae*	-	KX447443
24	*Orseolia oryzae*	-	KX447434
25	*Orseolia oryzae*	-	KM894412
26	*Culex quinquefasciatus*	-	GF112171

Table 4.12 The accession numbers of *O. oryzae* mitochondrial partial genome CO I sequences generated in this study

S. No	Samples	Place of collection	Accession number
1	Kadapa	Utukur	OR141889
2	Nellore-I	Vellukadu	OR142658
3	Nellore-II	Gadelladinne	OR122465
4	Chittoor-I	Vedam	OR143373
5	Chittoor-II	Peruru	OR241367

The present study is in agreement with the research conducted by Atray *et. al*. (2015) where the phylogenetic analysis was performed with 26 arthropods by utilizing mitogenome sequences. Based on the study, the genetic relationship of different gall midge populations collected from different locations of Southern zone was established through phylogenetic analysis. Separate clusters for gall midges of India, Thailand and *Culex quinquefasciatus* (mosquitos) which is agreement with the work done by Atray *et. al*. (2015).

Kattali *et. al*. (2015) conducted phylogenetic analysis of *Orseolia oryzae* and other species belongs to Cecidomyiidae family conducted a phylogenetic analysis using the COI sequence. The COI sequence analysis provided a clear insight into the genetic structure and phylogenetic status of *O. oryzae*. Specific nucleotides present at particular locations within the COI sequence allowed for the differentiation of *O. oryzae* from other species in the Cecidomyiidae family.

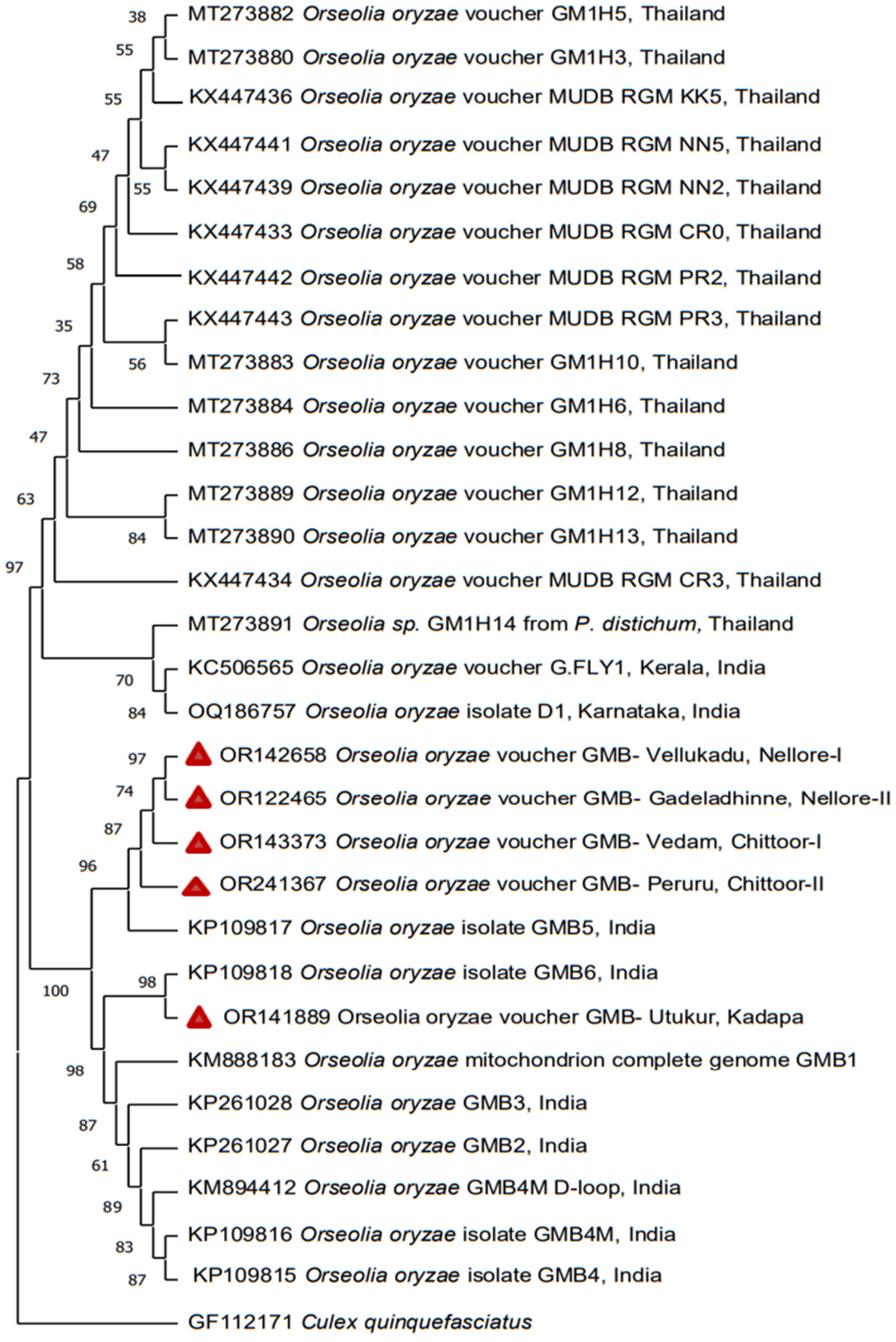


Fig. 4.3: Phylogenetic tree of *Orseolia oryzae* showing relationship of mtCOI gene sequences (600 bp) collected from different districts of Southern zone of Andhra Pradesh, India.

4.3 SCREENING OF RICE GENOTYPES AGAINST RICE GALL MIDGE IN Y.S.R. DISTRICT.

The 50 rice genotypes (including one resistant and one susceptible) against rice gall midge were evaluated under field condition at Agricultural Research Station, Utukur, Kadapa, Y.S.R District during late *kharif,* 2022 (Table 4.13). Rice gall midge was recorded as per cent mean plant damage (PD%) and per cent mean silver shoot (SS%) damage at 30 and 50 DAT. Since, the main objective of the study was to find the resistant genotypes, grading of genotype was done based on the data recorded at 50 DAT ensuring that the damage in the susceptible check (TN-1) was high.

The frequency of genotypes under different mean per cent levels of rice gall midge silver shoot and plant damage, as a reflection of pest pressure and performance of genotypes at ARS, Utukur, Kadapa at 30 and 50 DAT is given in Table 4.9 and fig 4.4 and 4.5.

4.3.1 Incidence of rice gall midge at 30 DAT in different rice genotypes

At 30 DAT, per cent mean silver shoot damage and plant damage ranged from 0.00 to 5.27 and 0.00 to 50.00 per cent among the entries. Out of all rice genotypes screened for the resistance to rice gall midge, sixteen genotypes were found to be highly resistant with zero silver shoot per cent, thirteen genotypes were found resistant, nineteen genotypes as moderate resistant, two genotypes as moderate susceptible and no genotype was found as susceptible and highly susceptible (Table 4.13). The nil silver shoot damage and plant damage was observed in the genotypes *viz.,* RP 4621 – 1845, RPE 924, RPE 937, RPE 938, RPE 940, RPE 259, RPE 272, RPE 1464, RPE 1564, RP 4621-1845, RP 4639-336, RP 4643-1025, RP 4092-365-117-10, DG-218, RP 4680-1-1-15 and PTB-33 were grouped under highly resistant genotypes (Score-0). The next lowest mean silver shoot damage of < 1.0 per cent was recorded in the genotype RP 4613-263, RPE 745, RPE 921, RPE 1034, RPE 1039, RPE 1042, RPE 1183, RPE 1205, RPE 1245, RPE 177, RPE 1387, RP-4639-179 and GEMP- 602 which have recorded 0.45, 0.24, 0.67, 0.82, 0.94, 0.90, 0.91, 0.45, 0.33, 0.40, 0.99, 0.71 and 0.73 per cent respectively and were categorized as resistant genotypes (Score-1). Highest

mean silver shoot damage was recorded in RPE 1385 about 5.27% and mean plant damage 50.00% was sorted as moderate susceptible genotype (Score-5). Remaining genotypes except the checks are grouped under moderate resistant genotypes (Score- 3) for rice gall midge.

Per cent mean silver shoot damage for rice gall midge in susceptible check (TN-1) recorded as 15.11% as susceptible (Score-7) and resistant check (PTB-33) 0.00% recorded as highly resistant (Score-0) at 30 DAT.

4.3.2 Incidence of rice gall midge at 50 DAT in different rice genotypes

At 50 DAT, per cent mean silver shoot damage and plant damage ranged from 0.00 to 15.11 and 0.00 to 75.00 per cent among the entries. Out of 50 genotypes screened for the resistance to rice gall midge, five genotypes were found to be highly resistant with zero silver shoot per cent, six genotypes were found resistant, 26 genotypes as moderate resistant, 11 genotypes as moderate susceptible and two genotype was found as susceptible and no genotype showed as highly susceptible (Table 4.13). The mean silver shoot damage and mean plant damage was observed in the genotypes *viz.,* RPE 937, RPE 1042, RPE 1183, RPE 1564 and GEP-548 which have recorded zero per cent were grouped under highly resistant genotypes (Score-0). The next lowest mean silver shoot damage was observed in the genotype RPE 939, RPE 1031, RPE 1034, RPE 1205, RPE 1249 and RPE 1845 which have recorded 0.66, 0.97, 0.98, 0.69, 0.72 and 0.73 per cent were categorized as resistant genotypes (Score-1). Next lower mean silver shoot damage was noticed in RPE 733, RPE 735, RPE 739, RPE 924, RPE 218, RPE 220, RPE 259, RPE 1154, RP-4639-461, RP-4643-1025 and RP-4680-1-1-15 genotypes expressing 6.66, 8.77, 6.32, 5.12, 7.08, 6.00, 6.26, 7.70, 9.70, 5.20 and 7.02 per cent respectively were sorted in moderate susceptible (Score-5). Remaining genotypes are grouped under moderate resistant genotypes (Score- 3) for rice gall midge. Highest mean silver shoot damage was recorded in RPE 1385 about 15.4 per cent was sorted in susceptible genotype (Score-7) and but mean plant damage was recorded in RPE 259 is 75.00 per cent.

Table 4.13 Reaction of rice genotypes to gall midge at ARS, Utukur, Kadapa during late *kharif*-2022.

S. No.	Genotypes	Designation	Mean Damage 30 DAT		Mean Damage 50 DAT		Scoring based on 30 and 50 DAT
			Silver Shoot SS (%) *	Plant Damage PD (%) *	Silver Shoot SS (%) *	Plant Damage PD (%) *	
1	DG-130	RP 4613-261	2.13 (7.93)	20.00 (25.82)	4.01 (11.55)	30.00 (32.90)	3
2	DG-131	RP 4613-263	0.45 (2.73)	5.00 (9.31)	2.00 (8.11)	25.00 (29.89)	3
3	DG-137	RP 4621 -1845	0.00 (0.00)	0.00 (0.00)	3.23 (10.36)	30.00 (32.90)	3
4	DG-144	RPE 733	2.02 (7.59)	25.00 (28.83)	6.66 (14.96)	55.00 (47.89)	5
5	DG-145	RPE 735	2.09 (7.57)	25.00 (28.83)	8.77 (17.22)	55.00 (47.89)	5
6	DG-148	RPE 739	1.22 (4.50)	10.00 (13.28)	6.32 (14.56)	45.00 (42.11)	5
7	DG-151	RPE 745	0.24 (1.99)	5.00 (9.21)	3.30 (10.46)	25.00 (29.89)	3
8	DG-153	RPE 920	1.82 (5.50)	15.00 (16.60)	2.05 (8.23)	30.00 (33.21)	3
9	DG-154	RPE 921	0.67 (4.70)	10.00 (18.43)	1.38 (6.76)	10.00 (18.43)	3
10	DG-155	RPE 924	0.00 (0.00)	0.00 (0.00)	5.12 (13.08)	40.00 (39.23)	5
11	DG-156	RPE 928	1.59 (7.22)	20.00 (26.56)	3.00 (9.77)	30.00 (33.21)	3
12	DG-157	RPE 937	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0

13	DG-158	RPE 938	0.00 (0.00)	0.00 (0.00)	1.07 (5.91)	25.00 (29.89)	3
14	DG-159	RPE 939	1.96 (5.71)	20.00 (19.61)	0.66 (4.61)	10.00 (18.43)	3
15	DG-160	RPE 940	0.00 (0.00)	0.00 (0.00)	1.64 (7.23)	15.00 (22.50)	3
16	DG-162	RPE 1031	1.10 (6.00)	15.00 (22.50)	0.97 (5.65)	10.00 (18.43)	3
17	DG-163	RPE 1034	0.82 (3.67)	10.00 (13.29)	0.98 (5.16)	25.00 (29.89)	1
18	DG-164	RPE 1039	0.94 (3.95)	10.00 (13.29)	1.55 (7.07)	30.00 (32.90)	3
19	DG-165	RPE 1042	0.90 (3.87)	15.00 (16.60)	0.00 (0.00)	0.00 (0.00)	**1**
20	DG-167	RPE 1183	0.91 (3.89)	10.00 (13.29)	0.00 (0.00)	0.00 (0.00)	**1**
21	DG-168	RPE 1205	0.45 (2.72)	5.00 (9.21)	0.69 (4.75)	10.00 (18.43)	1
22	DG-170	RPE 1245	0.33 (2.32)	5.00 (9.21)	1.67 (7.22)	40.00 (39.10)	3
23	DG-171	RPE 1249	1.47 (6.63)	20.00 (25.82)	0.72 (4.86)	10.00 (18.43)	**3**
24	DG-173	RPE 1257	3.37 (10.26)	35.00 (35.79)	3.87 (11.34)	50.00 (45.00)	3
25	DG-174	RPE 1258	2.45 (8.90)	25.00 (29.89)	3.08 (10.11)	40.00 (39.23)	3
26	DG-176	RPE 1261	1.85 (5.54)	20.00 (19.61)	1.08 (5.95)	15.00 (22.50)	3
27	DG-178	RPE 69	2.02 (7.52)	30.00 (31.71)	1.77 (7.63)	25.00 (29.89)	3

28	DG-180	RPE 99	1.45 (4.91)	15.00 (16.60)	3.41 (10.65)	35.00 (36.22)	3
29	DG-181	RPE 177	0.40 (2.57)	5.00 (9.21)	1.85 (7.80)	25.00 (29.89)	3
30	DG-182	RPE 218	1.91 (7.95)	25.00 (29.89)	7.08 (15.44)	65.00 (53.78)	5
31	DG-183	RPE 220	2.91 (8.52)	25.00 (29.89)	6.00 (14.18)	45.00 (42.11)	5
32	DG-184	RPE 259	0.00 (0.00)	0.00 (0.00)	6.26 (14.49)	75.00 (60.11)	5
33	DG-185	RPE 272	0.00 (0.00)	0.00 (0.00)	3.85 (11.30)	25.00 (29.89)	3
34	DG-187	RPE 1154	1.35 (6.66)	15.00 (22.50)	7.70 (16.10)	35.00 (36.22)	5
35	DG-189	RPE 1385	5.27 (13.26)	50.00 (45.00)	15.11 (22.87)	55.00 (47.89)	7
36	DG-190	RPE 1387	0.99 (5.67)	15.00 (22.50)	2.36 (8.69)	20.00 (26.57)	3
37	DG-191	RPE 1464	0.00 (0.00)	0.00 (0.00)	2.27 (8.66)	25.00 (29.89)	3
38	DG-192	RPE 1564	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0
39	DG-198	RP 4621-1845	0.00 (0.00)	0.00 (0.00)	0.73 (0.00)	10.00 (18.43)	1
40	DG-200	RP 4639-179	0.71 (4.80)	15.00 (22.50)	2.48 (9.06)	30.00 (32.90)	3
41	DG-201	RP 4639-336	0.00 (0.00)	0.00 (0.00)	4.60 (12.35)	40.00 (39.10)	3
42	DG-202	RP 4639-461	3.49 (10.76)	30.00 (32.89)	9.70 (18.14)	75.00 (60.11)	5

43	DG-204	RP 4643-1025	0.00 (0.00)	0.00 (0.00)	5.20 (13.18)	40.00 (39.28)	5
44	DG-211	GEMP-548	1.25 (6.41)	20.00 (26.56)	0.00 (0.00)	0.00 (0.00)	**3**
45	DG-212	GEMP-602	0.73 (4.89)	10.00 (18.43)	2.99 (9.94)	20.00 (26.56)	3
46	DG-217	RP 4092-365-117-10	0.00 (0.00)	0.00 (0.00)	4.43 (12.12)	30.00 (33.21)	3
47	DG-218	-	0.00 (0.00)	0.00 (0.00)	4.05 (11.58)	30.00 (33.21)	3
48	DG-221	RP 4680-1-1-15	0.00 (0.00)	0.00 (0.00)	7.02 (15.36)	60.00 (50.77)	5
49	TN-1 (check)	-	15.11 (22.87)	50.00 (45.00)	27.89 (31.88)	90.00 (76.71)	9
50	PTB-33 (check)	-	0.00 (0.00)	0.00 (0.00)	2.97 (9.91)	30.00 (33.21)	3
	Grand Mean		1.33	12.60	3.87	30.80	
	F- value		2.54	2.45	66.36	19.31	
	P- value (0.05)		0.001	0.001	0.0001	0.0001	
	Significant		**S**	**S**	**S**	**S**	

Note: DAT – Date After Transplantation, * Transformation – Arcsine transformation.

Table 4.14 List of ranges of per cent mean Silver Shoot damage at 30 and 50 DAT

Score	Reaction	Mean silver shoot damage per cent (SS%)	30DAT	50DAT
0	Highly Resistant	0	16	5
1	Resistant	<1%	13	6
3	Moderately Resistant	1-5%	19	26
5	Moderately Susceptible	6-10%	1	11
7	Susceptible	11-25%	1	1
9	Highly Susceptible	>25%	-	1

Table 4.15 List of ranges per cent mean plant damage at 30 and 50 DAT

Mean Plant Damage (PD%)	30 DAT	50 DAT
0-10%	27	11
11-20%	13	4
21-30%	7	16
31-40%	1	8
41-50%	2	3
51-60%	-	4
61-70%	-	1
71-80%	-	2
81-90%	-	1
90-100%	-	-

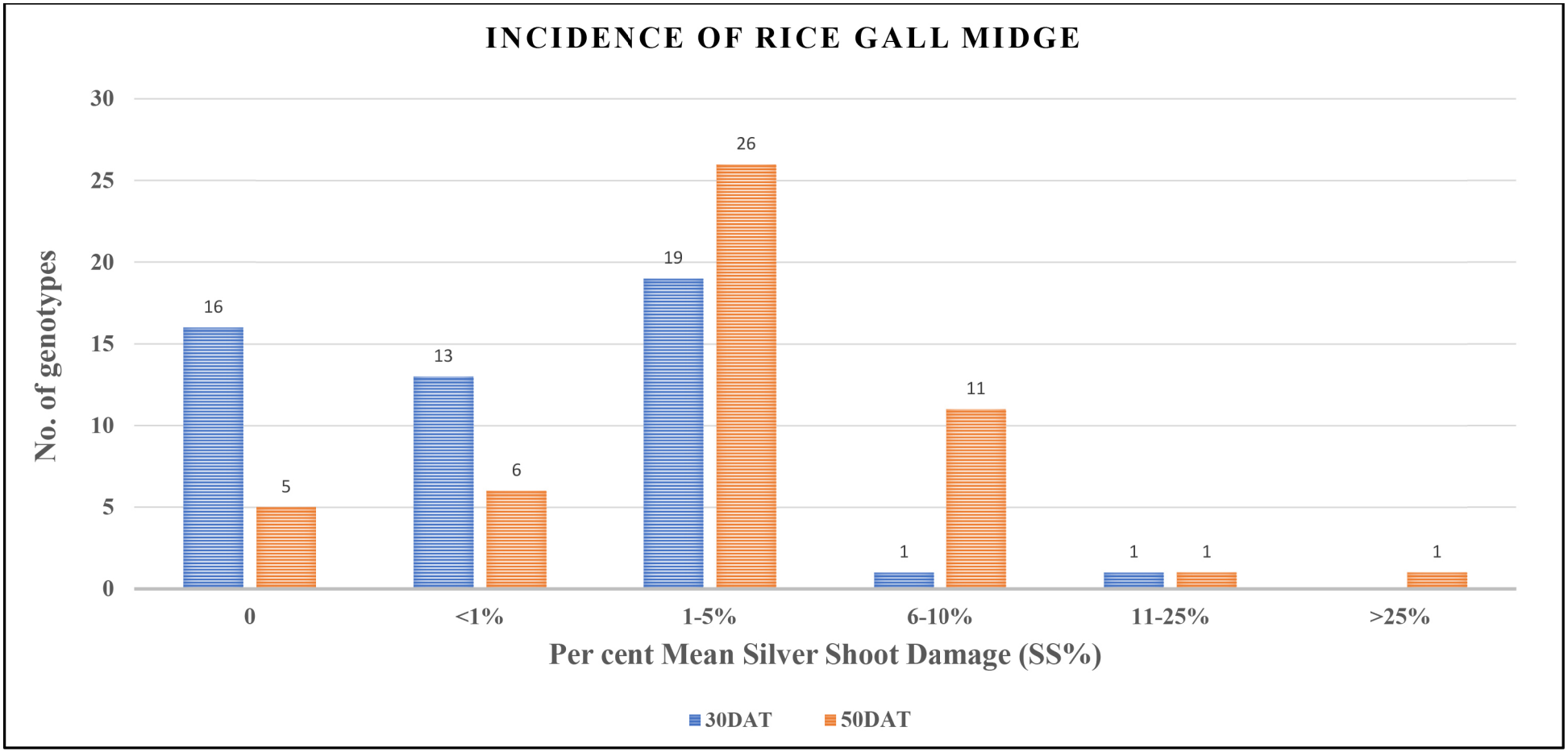


Fig 4.4: Frequency distribution of SS% reaction of rice genotypes against gall midge at ARS, Utukur, Kadapa

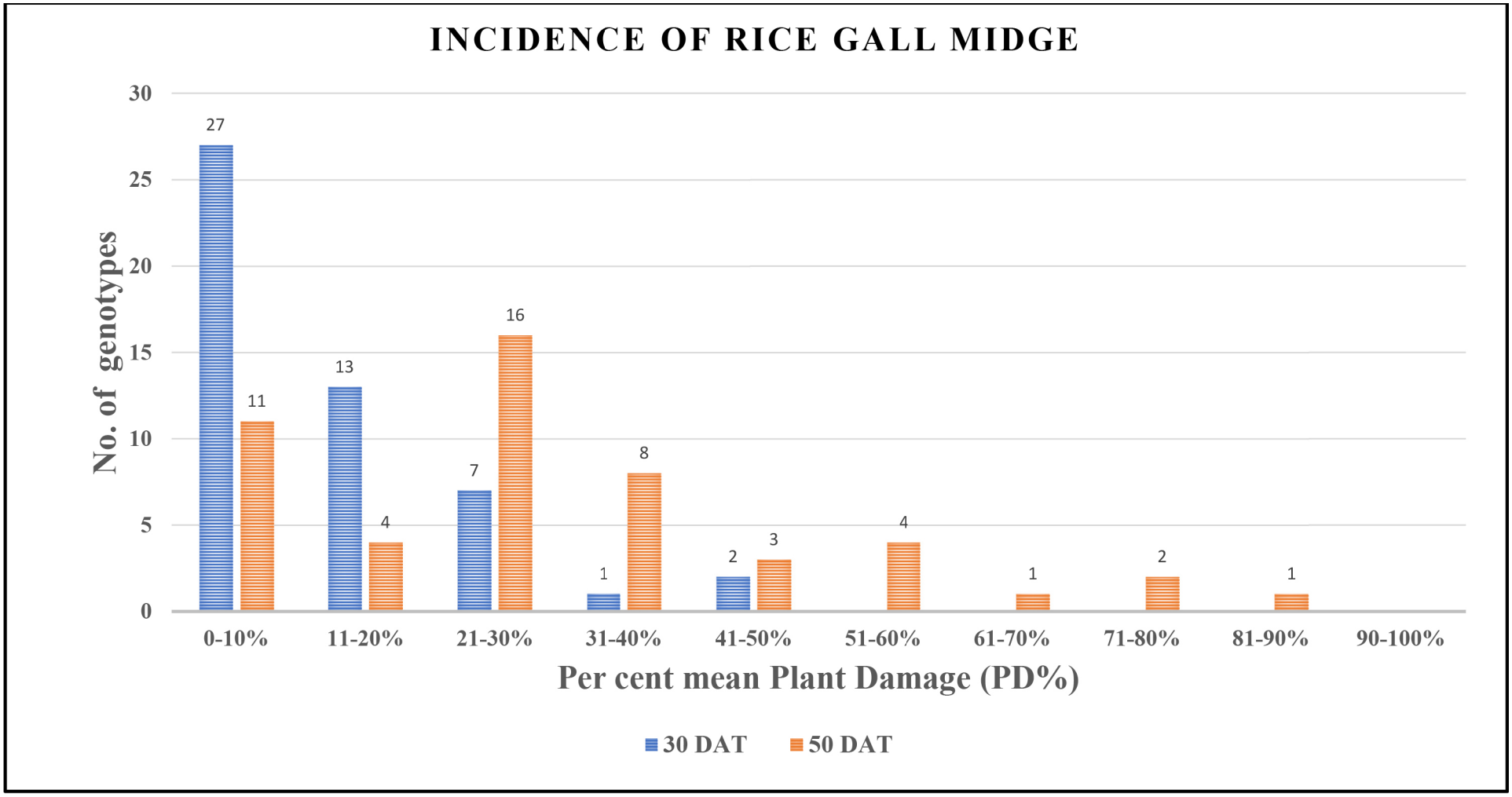


Fig 4.5: Frequency distribution of PD% reaction of rice genotypes against gall midge at ARS, Utukur, Kadapa

Table 4.16 Grouping of rice genotypes in screening trial against gall midge by using Standard Evaluation (IRRI 2002) at 30 DAT

Per cent damage	Score	Reaction	Name of the genotypes/ Designation	Total No. of genotypes/ Designation
0	0	Highly Resistant	RP 4621-1845, RPE 924, RPE 937, RPE 938, RPE 940, RPE 259, RPE 272, RPE 1464, RPE 1564, RP 4621-1845, RP 4639-336, RP 4643-1025, RP 4092-365-117-10, DG-218, RP 4680-1-1-15 and PTB-33	16
<1	1	Resistant	RP 4613-263, RPE 745, RPE 921, RPE 1034, RPE 1039, RPE 1042, RPE 1183, RPE 1205, RPE 1245, RPE 177, RPE 1387, RP-4639-179 and GEMP- 602	13
1-5	3	Moderately Resistant	RP 4613-261, RPE 733, RPE 735, RPE 739, RPE 920, RPE 928, RPE 939, RPE 1031, RPE 1249, RPE 1257, RPE1258, RPE 1261, RPE 69, RPE 99, RPE 218, RPE 220, RPE 1154, RP-4639-461 and GEMP-548	19
6-10	5	Moderately Susceptible	RPE 1385	1
11-25	7	Susceptible	TN-1	1
>25	9	Highly Susceptible	-	0

Table 4.17 Grouping of rice genotypes in screening trial against rice gall midge by using Standard Evaluation (IRRI 2002) at 50 DAT

Per cent damage	Score	Reaction	Name of the genotypes / Designation	Total No. of genotypes / Designation
0	0	Highly Resistant	RPE 937, RPE 1042, RPE 1183, RPE 1564 and GEP-548	5
<1	1	Resistant	RPE 939, RPE 1031, RPE 1034, RPE 1205, RPE 1249 and RPE 1845	6
1-5	3	Moderately Resistant	RP 4613-261, RP 4613- 263, RP 4621-1845, RPE 745, RPE 920, RPE 921, RPE 928, RPE 938, RPE 940, RPE 1039, RPE 1245, RPE 1257, RPE 1258, RPE 1261, RPE 69, RPE 99, RPE 177, RPE 272, RPE 1387, RPE 1464, RP-4639-179, RP- 4639-336 GEMP-602, RP-4092-365-117-10, DG-218 and PTB-33	26
6-10	5	Moderately Susceptible	RPE 733, RPE 735, RPE 739, RPE 924, RPE 218, RPE 220, RPE 259, RPE 1154, RP-4639-461, RP-4643-1025 and RP-4680-1-1-15	11
11-25	7	Susceptible	RPE 1385	1
>25	9	Highly Susceptible	TN-1	1

Per cent mean silver shoot damage for rice gall midge in susceptible check (TN-1) recorded as 27.89 per cent susceptible (Score-9) and resistant check (PTB-33) 2.97 per cent recorded as moderate resistant (Score-3) at 50 DAT.

From the study, two common rice genotypes that showed highly resistant at both DAT's were RPE 937 and RPE1564. They can be used as donors in resistant breeding trials at ARS, Utukur, Kadapa.

The present study revealed that all the genotypes recorded higher per cent silver shoot damage at 50 DAT than the 30 DAT. This finding supported by Malathi and Shravan Kumar *et. al*. (2021) reported peak silver shoot damage at 50 DAT for WGRH18 (38.14), JGLH 275 (31.08), JGL 30090 (23.08) AND WGL 825 (20.12). Anusha *et. al*. (2022) showed high damage at 50 DAT for IR 79216-141-1-3-3-3 (45.0) and JGL 11470 (15.0). Meher *et. al*. (2009) reported that highest silver shoot damage in susceptible check TN-1 genotype.

Anil *et. al*. (2022) reported that, out of 84 rice germplasm lines tested, seven entries were found highly resistant to biotype 3 (GMB 3) at Regional Agricultural Research Station (RARS), Jagtial. Vinita *et. al.* (2023) observed that out of 115 genotypes screened, 28 entries showed 'nil' damage and found highly resistance to rice gall midge.

The present study also showed that resistant check (PTB-33) also has the incidence of rice gall midge at 50 DAT about 2.97 per cent silver shoot damage and 30.00 per cent of plant damage. This finding supported by Anusha *et. al*. (2022) reported that the resistant check (RP 2068-18-3-5) have the incidence on both 30 and 50 DAT about 3.00 and 7.40 per cent of silver shoot and 14.20 and 16.6 per cent of plant damage at RARS, Warangal.

The study identified two rice genotypes, RPE 937 and RPE 1564, which exhibited high resistance at both DAT's. These genotypes can serve as valuable donors in resistance breeding trails to be conducted at ARS, Utukur, Kadapa.

Chapter - V

Summary & Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

5.1 SUMMARY

Rice gall midge (RGM), *Oresolia oryzae* is the most important pest in the paddy crop that devastated the paddy crop in different South Indian states. Many major rice growing districts of Andhra Pradesh were also severely affected by this insect. Rapid roving survey was conducted in all three rice growing districts of Southern zone of Andhra Pradesh to know the gall midge incidence and severity of plant damage. Maggots feed and live inside the silver shoot, most of the insecticides are not does not control rice gall midge. Majorly, the granular insecticidal applications in the early stage of the rice crop and during tillering stage would protect the crop from gall midge.

During survey conducted for RGM infestation in three districts of Southern of Andhra Pradesh, Nellore District recorded highest mean silver shoot 15.38 per cent followed by Y.S.R District of 12.19 per cent and lowest recorded in Chittoor District about 4.50 per cent. Y.S.R District recorded highest mean plant damage (76.00 %) followed by Nellore District (71.20 %) and Chittoor District (49.92 %).

The higher incidence of gall midge in Nellore District, particularly in Vellukadu village, can be attributed to the lack of awareness about the latest chemicals for controlling the pest. Instead, they are relying on conventional insecticides like neem formulations, which are not effective against RGM. In the village of Gadeladhinne, where the NLR-34449 variety is cultivated, there is a noticed high incidence of RGM cases compared to other varieties. This could be due to late transplantation practices and the presence of favorable environmental conditions for RGM infestation. Y.S.R District was recorded as medium incidence of RGM may be due to the application of granular insecticides in the early stage of crop to manage the RGM and less favorable abiotic factors to RGM when compared to Nellore District rice farmers Andhra Pradesh. Chittoor District was recorded as lower incidence compare to other two districts may be due to the growing of resistance rice cultivars and perfect stage of application of granular insecticides to manage to RGM.

These results highlight the variability in rice gall midge infestation levels across the surveyed districts in Andhra Pradesh during the *kharif* and *rabi* seasons of 2022. The data obtained from this survey will aid in implementing targeted measures to manage and mitigate the impact of rice gall midge on rice production in the affected areas.

17 differentials (ARS, Ragolu) and 16 differentials (IIRR, Hyderabad) were transplanted in different dates (11-08-2022, 23-09-2022 and 13-10-2022) at ARS, Utukur, Kadapa. Data obtained from all entries revealed that reaction pattern observed against the existing biotype in Y.S.R District was not following the perfect reaction pattern. In all the three dates of transplantation the resistant entries recorded were W 1263, ARC 6605 from group I, ARC 5984 from group II and finally INRC 3021 and AGANNI from group IV. The research study revealed that the biotype existing in Y.S.R District was following the reaction nearer to the biotype VI with percent similarity index (PSI) of 76.47 per cent. Highest incidence of gall midge was observed in ABHAYA (4.71%) at first date of transplantation, PHALGUNA (29.63%) at second date of transplantation and INRC 15888 (23.84%) at third date of transplantation.

Molecular studies were carried out by using gall midge samples collected from three districts of Southern zone in Andhra Pradesh to determine the existing biotypes in the respective geographical areas. The molecular analysis was conducted at the molecular biology laboratory of the Institute of Frontier Technology located in RARS, Tirupati. The promising technique utilized in the present study was Polymerase Chain Reaction (PCR) amplification with SSR, SCAR and mtCOI primers. The SSR primers were used to identify three distinct biotypes (GMB1, GMB4 and GMB4M) from five samples Chittoor I (Vedam), and Chittoor II (Peruru), Nellore I (Vellukadu), Nellore II (Gadeladhinne), Kadapa (Utukur). The band fragment sizes observed with primers Oosat21, Oosat35 and Oosat43 matched the expected ranges for biotypes 1, 4 and 4M, respectively, suggesting their correspondence. However, the Kadapa sample did not produce an amplified band with the Oosat35 primer. Different primers (Oosat16, Oosat24 and Oosat59) yielded band fragment sizes that significantly differed from the expected sizes for GMB1, GMB4 and GMB4M, indicating their unsuitability for

identification. Overall, the study found a mixed population of biotypes in the five samples.

The SCAR markers Y132 and Y133 were found to be specific for biotype 5 and biotype 4, respectively. The band fragment sizes associated with these markers were approximately 600bp and 750bp. Using SCAR marker Y132, DNA samples from Nellore I (Vellukadu), Nellore II (Gadeladhinne), Chittoor I (Vedam) and Chittoor II (Peruru) were successfully amplified. The resulting fragment lengths precisely matched the expected band fragment size of 600bp, providing clear evidence that these samples belong to biotype 5. However, it is important to note that the Kadapa (Utukur) sample did not undergo amplification with SCAR marker Y132, indicating that it does not belong to biotype 5. All five DNA samples, were subjected to amplification with Y133 SCAR marker at an annealing temperature of 55°C. However, the observed band fragment size for all five samples was 1000bp, which significantly differed from the expected band fragment size of 750bp for biotype 4. This discrepancy suggests that none of the five samples belong to biotype 4 and raises the possibility of the presence of other biotypes within the population.

Phylogenetic analysis, which examines the evolutionary relationships among organisms, was considered an essential tool for effective identification biotype. The phylogenetic tree construction revealed the presence of two distinct clades. The first clade included biotypes of *O. oryzae* identified as GMB1, GMB2, GMB3, GMB4, GMB4M, GMB6, and the Kadapa sample. The second clade consisted of GMB5 and other samples from the Southern zone, specifically Nellore-I, Nellore-II, Chittoor-I and Chittoor-II. In this context, it is worth mentioning that the genetic groups Nellore-I (OR142658) and II (OR122465), as well as Chittoor-I (OR241367) and II (OR143373) of the present study, showed significant bootstrap support (96%) with *O. oryzae* population GMB5 (KP109817) from India. Similarly, the genetic group Utukur, Kadapa (OR141889) exhibited substantial bootstrap support (98%) with *O. oryzae* population GMB6 (KP109818) of India. These findings indicate a close genetic relationship between the mentioned genetic groups and their corresponding *O. oryzae* populations from India.

Field evaluation of 50 genotypes including one resistant and one susceptible check was conducted at ARS, Utukur, Kadapa, Y.S.R District during the late *kharif* season of 2022. The objective of the evaluation was to assess the response of 50 rice genotypes to rice gall midge infestation. At 30 DAT, 16 rice genotypes exhibited resistance to gall midge with a score of 0. However, at 50 DAT, only five rice genotypes, namely RPE 937, RPE 1042, RPE 1183, RPE 1564 and GEP-548, displayed with score of 0. The study identified two common rice genotypes, RPE 937 and RPE 1564, which exhibited high resistance at both 30 and 50 DAT. These genotypes can serve as valuable donors in resistance breeding trials to be conducted at ARS, Utukur, Kadapa.

5.2 CONCLUSION

- The survey conducted across the three districts of Southern zone in Andhra Pradesh during *kharif* and *rabi*, 2022, revealed that Vellukadu village, Sullurupeta mandal, Nellore District, recorded the highest silver shoot incidence in the variety NLR-4001. While in Cherlopalle village of Sri kalahasti mandal, Chittoor District, the survey recorded the lowest silver shoot incidence in the variety RNR-15048 was recorded. This occurrence was attributed to the varieties used, abiotic factors that promoted gall midge multiplication and also time of insecticidal application.

- The biotype identification trials revealed a close resemblance of the reaction pattern observed in Kadapa to biotype VI on all three dates of transplantation at ARS, Utukur, Kadapa. The Per cent Similarity Index was 76.47%, indicating a significant similarity with biotype VI.

- The molecular identification of rice gall midge biotypes in Southern zone Andhra Pradesh, showed that the Kadapa (Utukur) biotype was distinct from Nellore I, Nellore-II, Chittoor I and Chittoor II, which all belonged to biotype V with help of Y132 SCAR marker. The mtCOI primer analysis indicated a close genetic resemblance of the Kadapa (Utukur) biotype to biotype VI, with a bootstrap value of 98. These insights are valuable for pest management and crop protection strategies.

- Field evaluation of 50 rice genotypes, including one resistant and one susceptible check, was conducted at ARS, Utukur, Kadapa, Y.S.R District during the late *kharif* season of 2022. Among the genotypes assessed, RPE 937 and RPE 1564 displayed high resistance to rice gall midge infestation at 30 and 50 DAT. These genotypes hold promise as valuable donors in resistance breeding trials to be conducted at ARS, Utukur, Kadapa.

FUTURE THRUST

- The identified genotypes RPE 937 and RPE 1564 should be evaluated for one more season or in green house can be explored for further genetic studies to identify the new sources of resistance.
- Continuous monitoring of biotype pattern of the local gall midge population is necessary to formulate new management strategies and to develop varieties with durable resistance against emerging gall midge biotypes.
- Exploring alternate hosts for the rice gall midge and devising holistic offseason management strategies.

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Websites:

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www.statista.com. 2020-21.

Appendices

APPENDIX-I

Survey Data Sheet

Survey for rice gall midge infestation in rice farmers

1. Name of reporter:

2. Serial number of the farmer:

3. Date of collection:

4. Name of the cultivar:

5. Locality:

    a. Village:

    b. Mandal:

    c. District:

6. Season of the crop:

7. Total cropped area:

8. Stage of the crop:

9. Condition of the crop:

a) Symptoms if any:

    i.

    ii.

b) Percentage of Silver shoot:

c) Percentage of Plant damage

10. Previous crop:

11. Soil type:

12. Other diseases:

14. Any other information:

(Plant protection chemicals, Fertilizer etc., applied)

APPENDIX-II

REAGENTS USED FOR TOTAL DNA ISOLATION AND AGAROSE GEL ELECTROPHORESIS

CTAB Extraction buffer preparation:

BUFFER STOCKS PREPARATION:

A. 1M Tris HCL preparation (100ml):

Dissolve 12.114 grams (if mol. Wt. is 121.14 gms/mol) or 15.76 gms (if mol.wt. is 157.6 gms/mol) of Tris HCL in 70 ml distilled water and finally make the volume up to 100ml.

Note: PH should be maintained at 8.

B. 5M NACL preparation (100ml):

Dissolve 29.22 grams (if mol. Wt. is 58.44 gms/mol) of NaCl in 70 ml distilled water and finally make the volume up to 100ml.

C. 0.5M EDTA preparation: -

Dissolve 14.61grams (if mol. Wt. is 292.24 gms/mol) or 18.612gms (if mol.wt. is 372.24 gms/mol) of EDTA in 70 ml distilled water and finally make the volume up to 100ml. PH should be maintained at 8. Hence during the preparation should be add NaOH pellets until it dissolves and then make up the volume to 100ml.

a) PROTEINASE K – (10mg/ml)

The lyophilised powder was dissolved at a concentration of 10 mg/ml in sterile water. The stock solution was divided into small aliquots and stored at -20°C. Each aliquot could be thawed and refrozen several times.

b) RNAase (10mg/ml)

The powder was dissolved at a concentration of 10 mg/ml in sterile water. The stock solution was divided into small aliquots and stored at -20°C. Each aliquot could be thawed and refrozen several times.

c) 1XTE Buffer

10mM Tris-HCl (pH7.6)

1mM EDTA (pH8.O)

It was made up to 100ml and the solution was sterilized by autoclaving.

d) 10XTBE (1 Litre)

Tris-base 107 gm.

Boric acid 53.5 gm.

0.5M EDTA 7.444gm.

The distilled water was added to one litre and the concentration of buffer was diluted just before use.

e) ETHIDIUM BROMIDE

Ten mg/ml of Ethidium bromide was dissolved in sterile water and the container was wrapped with aluminium foil and stored at 4°C.

APPENDIX-III

Orseolia oryzae voucher GMB- Gadeladinne-2023 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: OR122465.1

FASTA Graphics

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  TITLE     Orseolia oryzae - mitochondrial cytochrome oxidase subunit-1
            (CO-1), partial genome
  JOURNAL   Unpublished
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  AUTHORS   Murali Krishna,M., Sunil Kumar,K., Manjula,K. and Harathi,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-JUN-2023) Department of Agriculture Entomolgy,
            ANGRAU, S.V AGRICULTURAL COLLEGE, NEAR STAFF QUATERS, TIRUPATI,
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Orseolia oryzae voucher GMB- KADAPA-2023 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: OR141889.1

FASTA Graphics

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  AUTHORS   Murali Krishna,M.
  TITLE     Orseolia oryzae - mitochondrial cytochrome oxidase subunit-1
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 457)
  AUTHORS   Murali Krishna,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-JUN-2023) Department of Agriculture Entomolgy,
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Orseolia oryzae voucher GMB- NELLORE -2023 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: OR142658.1

FASTA Graphics

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Orseolia oryzae voucher GMB- VEDAM -2023 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: OR143373.1

FASTA Graphics

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  TITLE     Orseolia oryzae - mitochondrial cytochrome oxidase subunit-1
            (CO-1), partial genome
  JOURNAL   Unpublished
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  TITLE     Direct Submission
  JOURNAL   Submitted (17-JUN-2023) Department of Agriculture Entomolgy,
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Orseolia oryzae voucher GMB- WET-2023 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial

GenBank: OR241367.1

FASTA Graphics

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  TITLE     Orseolia oryzae - mitochondrial cytochrome oxidase subunit-1
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  JOURNAL   Unpublished
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  AUTHORS   Murali Krishna,M., Sunil Kumar,K., Manjula,K. and Harathi,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-JUL-2023) Department of Agriculture Entomolgy,
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      421 catattgcag gaatttcttc tattttagga gctatcaatt ttatttcaac catattaaat
      481 ataaaaatta aattcctaaa atttgatcaa atttcattat ttatttgatc aattttaatt
      541 actacaattt tattattatt atctttacca gtactagcag gagcaattac tatacttctt
      601 actg
//
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