Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2020; 8(1): 378-382

Received: 04-11-2019
Accepted: 06-12-2019

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Studies on genetic variability of gall midge populations Across Andhra Pradesh and Telangana using RAPD

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Abstract
Rapid Amplified Polymorphic Primers were used to assess the genetic diversity of gall midge population across Andhra Pradesh and Telangana. Three distinct groups were obtained in the cluster analysis, performed by the UN weighted pair-group method (UPGMA). The Gall midge population in Chittoor showed a closer genetic relation with the gall midge population from Nellore with a similarity coefficient of 0.75, while the population from Warangal was placed in second cluster. The population from Jagtial was placed in separate cluster indicating that it is distantly related to the gall midge populations in other two clusters.

Keywords: Rice gall midge, genetic diversity, RAPD

1. Introduction
Rice is staple food of South and South East Asian Countries. India is the second largest producer of Rice which is cultivated in an area of 42.7 million hectare (mha) with a total production of 161.27 million tons (mt) (IRRISTAT, 2017) [4]. More than 100 insects were reported to cause damage to rice crop (Pathak and Khan) [8]. Among them, only few are causing alarming damage to rice crop especially rice stem borer, leaf folder, gall midge and plant hoppers in Andhra Pradesh. Cultivating varieties with considerable amount of resistance against insects for many years resulted in emergence of biotypes with varying genetic constitution making the so-called resistant varieties susceptible. Likewise, rice gall midge biotype 3 was reported at Jagtial and Vijaya Lakshmi *et al.* (2006) [9] reported the occurrence of a new biotype 4M in the field evaluations using differentials at Warangal. As a result a total of seven biotypes of Asian rice gall midge were identified in India. In the recent years, rice gall midge has become a potential pest in rice growing areas of Southern Zone of Andhra Pradesh. Consistent reports are lacking on the existence of a particular rice gall midge biotype through conventional evaluation of rice differentials in Southern zone. Hence, an attempt was made to study the extent of genetic variation in gall midge populations of Chittoor, Nellore districts in Andhra Pradesh and of Jagtial and Warangal in Telangana using RAPD technique.

2. Materials & Methods
2.1 Collection of Insects
Plants infested with rice gall midge were collected from farmer's fields and planted in caged pots. The adults emerged in the evening were collected the next day and stored in 70% ethanol at -20ºC till DNA extraction.

2.2 Location
Insects were collected from Tirupati of Chittoor district, Nellore in Andhra Pradesh and from Warangal and Jagtial in Telangana.

2.3 Extraction Procedure
Genomic DNA from single insect was extracted following Behura *et al.* (1999) [3] with minor modifications.

2.4 Purity Check of DNA Isolated
The purity of isolated genomic DNA was estimated by agarose gel electrophoresis (1.0% agarose). The gel was visualized in Ultra violet light transmitted gel documentation system. After quality checking, the DNA samples were diluted (50 ng/µl) for Polymerase Chain Reaction (PCR) analysis.

2.5 PCR
The genomic DNA of gall midge was subjected to PCR amplification. PCR was carried out using thermo cycler (Bio-Rad, India). The PCR conditions used were as follows:

2.5.1 PCR Conditions for RAPD Primers

Table 1: Show the temperature and time

		Temperature	Time
Initial Denaturation	:	94 ºC	2 min
Cycle Denaturation	:	94 ºC	1 min
Annealing	:	37 ºC	1 min
Extension	:	72 ºC	2 min
Final Extension	:	72 ºC	7 min
Cooling	:	4 ºC	α
No. of cycles	:	45	

Table 2: The PCR conditions and volume

PCR Components		Volume
2x PCR Master mix	:	10.0 µl
Template DNA	:	3.0 µl
Forward Primer	:	1.0 µl
Reverse Primer	:	1.0 µl
Water	:	5.0 µl
Total	:	20.0 µl

2.6 Agarose Gel Electrophoresis and gel documentation
- Gel electrophoresis was carried out in 2% gel at a constant voltage of 150 volts for 2.0 hr with 1 kb DNA ladder (SM1343-Thermo Scientific, USA) as size reference standard.
- The gels were visualized in UV gel documentation system (Bio-Rad Laboratories, Inc., Berkeley, California) for analysis of DNA bands. The banding pattern was 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, California) with 1 kbp DNA ladder.

2.7 Statistical Analysis
Data matrix was generated in which all observed bands were listed. The RAPD pattern of each Biotype was evaluated by assigning '0' for the absence of band and '1' to all the reproducible and detectable bands in the gel and calculated Jaccard's similarity co-efficient for each pair-wise comparison. The biotypes were grouped based on their overall similarities. The similarity coefficients were calculated *insilico* following Jaccard (1908) [6], using the following formula.

Similarity coefficient = a/n

Where
a = Number of matching bands for each pair of comparisons
n = Total number of bands observed in two samples

The similarity coefficients were subjected to Un weighted Pair-Group Method of Arithmetical averages (UPGMA) cluster analysis for grouping the gall midge populations. Statistical Package for Social Sciences (SPSS 20.0) package was used for dendrogram preparation and cluster analysis.

3. Results and Discussion
RAPD markers have become the common yard sticks to measure genetic differences between individuals, within and between related species or population (Subhodh *et al.*, 2010) [10]. Molecular characterization of rice gall midge is considered to be consistent method to know the polymorphism and genetic variability among the populations from different locations. Hence, gall midge populations collected from Nellore and Chittoor districts in Andhra Pradesh; Warangal and Jagtial districts from Telangana were subjected to RAPD technique to study the level of diversity and to establish genetic similarities among them.
Total genomic DNA from single adult female gall midge collected from four locations was extracted. The quantity and quality of DNA was analyzed by running 2 µl of each sample in agarose gel. The DNA samples of rice gall midge from all locations produced clear, sharp and high molecular weight band in 2.0 per cent agarose gel.
Thirty random primers *viz*., OPA 1, OPA 5, OPA 7, OPA 8, OPA 13, OPA 14, OPA 15, OPB 3, OPB 7, OPB 9, OPC 2, OPC 8, OPC 15, OPE 1, OPE 2, OPE 4, OPE 6, OPE 8, OPE 15, OPM 1, OPM 3, OPM 5, OPM 12, OPM 18, OPM 20, OPR 2, OPR 18, OPAB 11, OPAB 18 and OPAB 11 were used for amplification of gall midges collected from different locations (Annie Diana Grace, 2004) [2] observed variation in genetic constitution of rice gall midge and grass (*cynodon dactylon*) gall when the DNA of was amplified with the RAPD primer, OPA 13. (Table 1).

3.1 Banding Pattern
Out of thirty primers, twenty-eight primers were used for PCR amplification which gave scorable and reproducible bands with high per cent polymorphism (68.36%) and with band size ranging from 0.2 kb to 2.0 kb. The present findings were in accordance with the findings of Behura *et al.* (1999) [3], where the fragment size of rice gall midge DNA isolated from different locations in India varied from 0.4 kb to 2.0 kb. Two primers *i.e.*, OPA 8 and OPAB 18 resulted in non-distinct amplification products and were discarded. A high degree of polymorphism was obtained with most of the primers especially with OPE-2 and OPM-20 with 24 bands each and OPB-10 with 5 bands which indicates wide variability in the gall midge populations. (Plate 1 and 2).
The polymorphic bands ranged from 5 to 24. A total of 452 bands were obtained with the above process, among which 309 bands were polymorphic (68.36%) in nature. In another study by Anbuselvi (2003) [1] the biotype specific fragments generated by the SCAR marker, Y13 (0.55 kb size) developed from RAPD could be able to distinguish the gall midge biotypes 1 and 6.
Banding profiles obtained with 28 primers for rice gall midge from 4 locations were analyzed on the basis of presence or absence of bands. Among the gall midge population from four locations, similarity coefficients were calculated to establish the genetic relationships and presented in Table 2. The similarity index values ranged from 0.41 to 0.75 which indicates the existence of genetic diversity at DNA level in rice gall midge populations across Andhra Pradesh and

Telangana. The most diverse pair was found to be Chittoor and Jagtial, with variation of 41 per cent whereas maximum closeness was observed between the population from Chittoor and Nellore with similarity coefficient of 0.75.

Table 3: Per cent Polymorphism among the rice gall midge populations across Andhra Pradesh and Telangana

Primer	No. of Polymorphic Bands	No. of Monomorphic bands	Total No. of bands	Per cent Polymorphism
OPA 1	6	0	6	100.00
OPA 5	10	2	12	83.33
OPA 7	2	5	7	28.57
OPA 13	12	2	14	85.71
OPA 14	7	12	19	36.84
OPA 15	19	4	23	82.61
OPA 3	10	0	10	100.00
OPB 7	10	2	12	83.33
OPB 9	13	9	22	59.09
OPC 2	18	5	23	78.26
OPC 8	13	7	20	65.00
OPC 15	9	12	21	42.86
OPE 1	8	0	8	100.00
OPE 2	6	18	24	25.00
OPE 4	19	0	19	100.0
OPE 6	18	4	22	81.82
OPE 8	15	0	15	100.0
OPE 15	3	9	12	25.00
OPM 1	16	6	22	72.73
OPM 3	4	1	5	80.00
OPM 5	11	5	16	68.75
OPM 12	10	7	17	58.52
OPM 18	9	3	12	75.00
OPM 20	15	9	24	62.50
OPR 2	7	8	15	46.67
OPR 18	12	4	16	75.00
OPAB 11	6	9	15	40.00
OPAB 12	21	0	21	100.00
Total	309	143	452	68.36

Table 4: Similarity coefficients among the Gall midge populations from different locations of AP and Telangana

Location	Chittoor	Nellore	Warangal	Jagtial
Chittoor	1.00			
Nellore	0.751	1.00		
Warangal	0.585	0.587	1.000	
Jagtial	0.412	0.460	0.586	1.00

3.2 Cluster Analysis

Similarity matrix was developed and relationships were evaluated by cluster analysis of data. The dendrogram was generated using paired matrix values by Unweighted Pair Group Method with Arithmetic mean (UPGMA). All the entries were grouped into 3 close knit clusters (Table 4 and Fig.1). Similar biodiversity studies using AFLP analysis of rice gall midge from five countries revealed that a total of 261 distinct AFLP bands were identified separating the rice gall midge populations into two distinct groups (Katiyar *et al.* (2000) [7]

Three major clusters were obtained after cluster analysis. The dendrogram of similarity coefficients of cluster I indicated that the gall midge populations from Chittoor and Nellore were genetically similar. In the cluster II, the gall midge population at Warangal is 58 per cent similar to population from Chittoor and 59 per cent similar to both Nellore and Jagtial. The Jagtial population in the cluster III is quite different from the other two clusters, and is showing the variation of 54 per cent with gall midge populations from Nellore. Isha Atray *et al.* (2015) [5] sequenced, annotated and analyzed the complete mitochondrial genome of all the seven Indian biotypes of Asian rice gall midge, *Orseolia oryzae* and observed that tandem repeats provide a valuable tool not only to distinguish the different biotypes of the gall midge. Molecular studies involving tandem repeats of mitogenome, AFLP and SCAR markers aids in identification of prevalent biotype in southern zone of Andhra Pradesh.

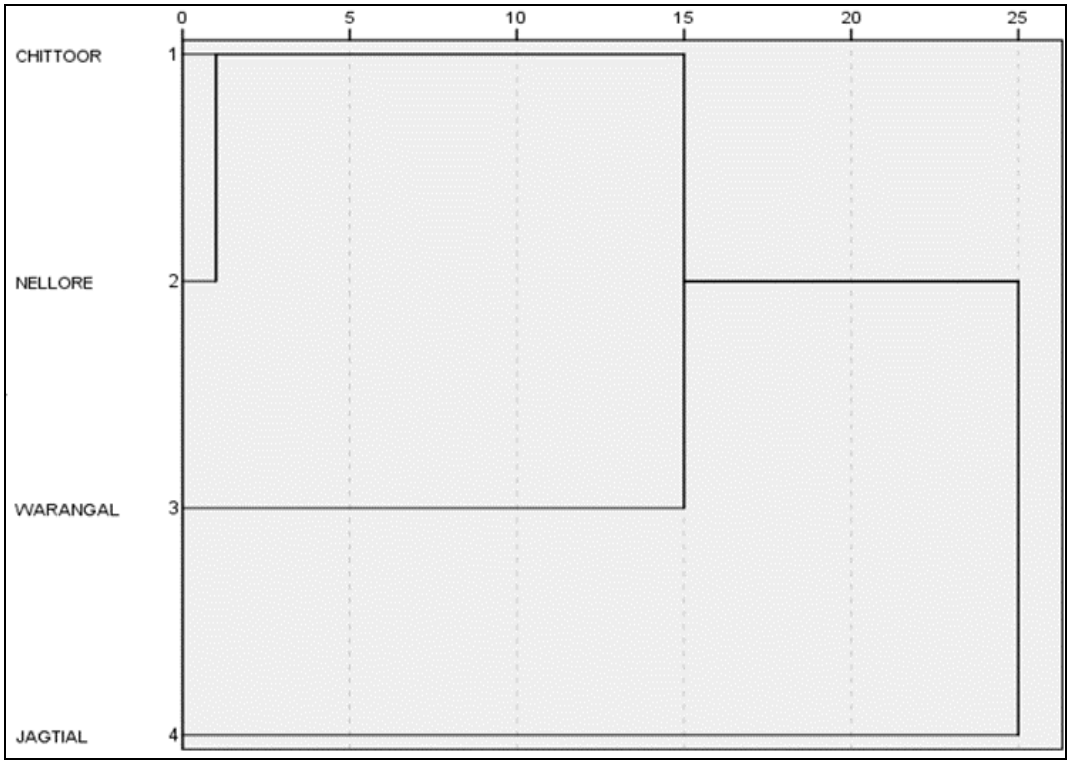

Fig 1: Dendrogram depicting the genetic variability in the rice Gall midge populations of Chittoor, Nellore, Warangal and Jagtial.

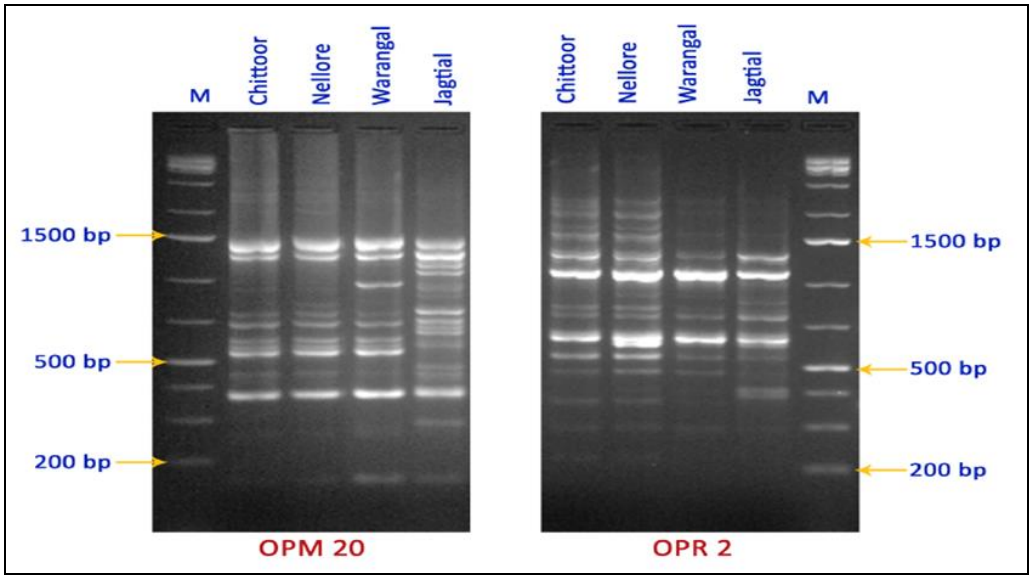

Plate 1: Genetic variability of rice gall midge populations across Andhra Pradesh and Telangana using OPM 20 and OPR 2 RAPD primers

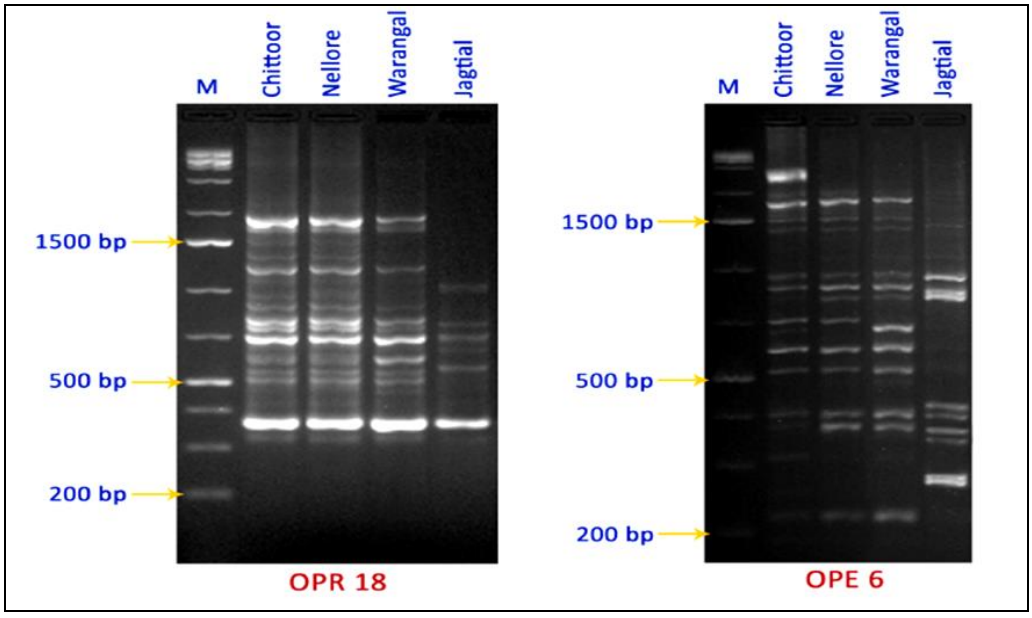

Plate 2: Genetic variability of rice gall midge populations across Andhra Pradesh and Telangana using OPR 18 and OPE 6 RAPD primers

4. Conclusion

In the present study on genetic variability of rice gall midge populations from various locations, highest per cent similarity of 75.0 was observed between the populations of Chittoor and Nellore and wide genetic variation between the populations of Chittor and Jagtial.

5. Acknowledgement

We thank Dr. S. Malathi, Principal Scientist (Entomology) Warangal, Dr. M. Venkataiah, Principal Scientist (Entomology) and Head, Regional Sugarcane and Rice Research Station, Rudrur, Dr. K. Devaki, Asst. Professor (Entomology), S.V. Agricultural College, Tirupati for the facilities and encouragement.

6. References

1. Anbuselvi. Virulence pattern and differentiation of asian rice gall midge, *Orseolia oryzae* (Wood-Mason) by sequence characterized amplified regions (SCARs). M.Sc. Thesis. Indira Gandhi Agricultural University, Raipur, 2003, 69.
2. Annie Diana Grace. Studies on Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) and its differentiation from *Cynodon dactylon* gall midge by RAPD Analysis Technique. M.Sc. Thesis. Indira Gandhi Agricultural University, Raipur, Chhattisgarh, 2004.
3. Behura SK, Sahu SC, Rajamani S, Devi A, Mago R, Nair Set *et al*. Differentiation of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason), biotypes by sequence characterized amplified regions (SCARs). Insect Molecular Biology. 1999; 8:391-397.
4. IRRISTAT, 2017. http://ricestat.irri.org:8080/wrsv3 /entrypoint.htm
5. Isha Atray, JS Bentur, Suresh Nair. The Asian Rice Gall Midge (*Orseolia oryzae*) mitogen me has evolved novel gene boundaries and tandem repeats that distinguish its biotypes. PLoS ONE, 10(7), e0134625.
6. Jaccard P. Nouvelles recherches surla distribution florale. Bulletin dela Société Vaudoise Des Sciences Naturelles. 1908; 44:223-270.
7. Katiyar S, Chandel, Girish, Tan Y, Zhang Y, Huang B *et al*. Biodiversity of Asian rice gall midge (*Orseolia oryzae*

Wood Mason) from five countries examined by AFLP analysis. Genome. 2000; 43:322-32.
8. Pathak Md, Khan Zr. Insect Pest of Rice. International Rice Research Institute, Manila, 1994, 2.
9. Vijaya Lakshmi P, Amudhan S, Hima Bindu K, Cheralu C, Bentur JS. A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. International Journal of Tropical Insect Science. 2006; 26(3):207-211.
10. Subhodh Kumar Jain, Bharat Neekhra, Divya Pandey, Kalpana Jain. RAPD Marker system in Insect Study: A review. Indian Journal of Biotechnology. 2010; 9:7-12.