Plant Archives Vol. 24, No. 2, 2024 pp. 2582-2588 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.370

INTRA POPULATION GENETIC DIVERSITY OF *SPODOPTERA FRUGIPERDA* IN RAYALASEEMA REGION OF ANDHRA PRADESH, INDIA

B.L. Manisha[1]*, N.C. Venkateswarlu[1], M.S.V. Chalam[1], R. Sarada Jayalakshmi Devi[2], B. Ramana Murthy[3], P. Lokesh[4] and M. Rafi[1]

[1]Department of Entomology, Acharya N.G. Ranga Agricultural University, Guntur - 517 502, Andhra Pradesh, India.
[2]Vice Chancellor, Acharya N.G. Ranga Agricultural University, Guntur - 522 034, Andhra Pradesh, India.
[3]Department of Statistics and Computer Application, Acharya N.G. Ranga Agricultural University, Guntur - 517 502, A. P., India.
[4]Department of Genetics and Plant Breeding, Acharya N.G. Ranga Agricultural University, Guntur - 517 502, Andhra Pradesh, India.
*Corresponding author E-mail : angraumanish2@gmail.com
(Date of Receiving-01-07-2024; Date of Acceptance-16-09-2024)

ABSTRACT

This study was conducted to check the variability of genetic structure within Fall armyworm populations of Rayalaseema region using RAPD markers —OPA-07, OPE-01, OPE-02, OPC-15 and OPM-01, which yielded polymorphic bands. The number of amplified bands ranged from 7 to 10, with OPA-07 producing the most loci (10) and OPE-01 the fewest (7). The size of the PCR products varied from 100 bp to 1500 bp. Polymorphism ranged from 66.66% (OPE-01) to 100% (OPA-07 and OPC-15) suggesting the existence of strong genetic polymorphism among FAW samples. The PIC values for the polymorphic markers ranged from 0.27 to 0.38. Statistical analyses as well Jaccard similarity index, showed significant genetic variations among the geographically distinct populations which could lead to difference in insecticide resistance levels These findings highlight the genetic diversity among Fall armyworm populations and validate the use of RAPD markers for assessing molecular variability.

Key words : *Spodoptera frugiperda*, RAPD markers, Polymorphism, Rayalaseema region.

Introduction

The fall armyworm, *Spodoptera frugiperda*, is a significant pest that poses a severe threat to agriculture across the globe. *S. frugiperda* has recently become the new invasive species in both the West and Central Africa where the outbreaks have been recorded for the very first time in early 2016 (Goergen *et al*., 2016). It was first noticed in the Indian subcontinent in Bangalore rural and Chikkaballapur districts during May and June 2018 (Ganiger *et al*., 2018 and Sharanabasappa *et al*., 2018) and South Karnataka during the first fortnight of July 2018 (ICAR-NBAIR pest alert, 2018). By 2019, FAW has been reported to attack Maize in Vietnam and Indonesia (Ginting *et al*., 2020). Within a year of pest introduction, it continued its spread across several countries in Asia (Kalleshwaraswamy *et al*., 2022).

In just 2 years since introduction, FAW has spread across all the Maize growing states of India, except the states of Himachal Pradesh and Jammu and Kashmir. In Andhra Pradesh, the pest was first reported in Vishakapatnam, Srikakulam and Vizianagaram districts during August, 2018 (Visalakshi *et al*., 2019).

The pest has become a major concern, particularly in Andhra Pradesh, where it has spread rapidly to every nook and corner of Rayalaseema region and it is inflicting substantial damage on staple crops such as maize, sorghum. Random Amplified polymorphic DNA (RAPD) markers offer a cost-effective and relatively straightforward approach for assessing genetic variation as well can provide insights into the genetic structure of local populations and reveal potential differences that might influence their behavior, or adaptability to environmental changes.

The study of genetic variability among *S. frugiperda*

populations in Rayalaseema using RAPD markers is crucial for several reasons. First, it can help in identifying genetic differences that may affect the pest's resistance to insecticides or biocontrol agents. Second, understanding population structure can guide the development of more effective and region-specific pest management strategies, this information contributes to the broader perspective of *S. frugiperda* genetics and evolution, which is essential for predicting and managing its spread and impact (Williams *et al*., 1990).

Understanding the genetic variability among populations of *S. frugiperda* is crucial for devising effective pest management strategies and developing sustainable control measures.

Materials and Methods

Larval populations were collected from Ramakuppam :12. 89031°N 78.54811°E, C.K. Dinne: 14.43252°N, 78.79145°E, Nandikotkur: 15.85725°N, 78.26624°E, Chilmattur: 13.840025°N, 77.703185°E ARS (Agricultural research station), ARS, Perumalpalli :13.3781°N 79.3243° E. From each geographical location, agriculture fields were surveyed and samples of FAW were collected by following W pattern (Davis and Williams scale, 1992). Healthy 5th instar larvae (six individuals) from each location were selected and were starved for 24 hr before DNA isolation. These were killed with Ethyl alcohol. All the samples were preserved in 99% ethanol and stored at -20^{0}C before DNA extraction.

DNA extraction

The molecular characterization studies of Fall armyworm larval populations obtained from different mandals of the Chittoor, Kadapa, Kurnool and Ananthapuramu districts were conducted at the Institute of Frontier Technology, RARS, Tirupati and S.V. Agricultural College, Tirupati. The collected larval population were subjected to molecular characterization by using modified CTAB (Cetyl Trimethyl Ammonium Bromide) protocol (Murry and Thompson, 1980).

Larvae were homogenized individually in pestle and mortar using 750 µL of extraction buffer. Then macerated sample was transferred to sterile Eppendorf tubes and added 2 ml of pre-heated (65°C) extraction buffer. The extraction buffer comprises of (1 M Tris (pH 8.0), 5 M NaCl, 0.5 M EDTA, 1% PVP and 0.1% Mercapto ethanol) and 2% CTAB (Cetyl Trimethyl Ammonium Bromide).

After homogenization, the extracts were transferred to 2 ml centrifuge tubes and kept for incubation at 65ºC for 1 hour in water bath (KEMI, KWB-220). After incubation the tubes were centrifuged in refrigerated centrifuge (Eppendorf, USA) at 10,000 rpm for 10 min at room temperature and the supernatant was collected into 2 ml fresh Eppendorf tubes.

Equal volume of phenol-chloroform (1:1 ratio) was added to the supernatant, mixed gently and later centrifuged at 10,000 rpm for 10 min and the supernatant was transferred to the 2 ml fresh Eppendorf tubes. Equal volumes of chloroform and isoamyl alcohol (24:1 ratio) were added and centrifuged at 10,000 rpm for 10 min. The supernatant was collected into separate Eppendorf tube and added with 0.6 volume of ice-cold isopropanol, 0.1 volume of sodium acetate (0.3M, pH 5.0) then incubated for 24 hours at -20°C.

After incubation, the tubes were taken out and centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was discarded and the pellet was washed with 70 per cent ethanol and again centrifuged at 13,000 rpm at 4°C for 10 min. After discarding the supernatant, the pellets were air dried and dissolved in 100 µL of sterile distilled water. The DNA samples were stored at -20°C in the deep freezer for further use.

Determining DNA Quality and Concentration

The concentration and quality of DNA was estimated using nano drop spectrophotometer at 260 nm (ND-2000C Spectrophotometer-Thermoscientific). Nanodrop spectrophotometric method was based on measuring the amount of ultraviolet (UV) irradiation that is absorbed by the bases. The ratio of OD 260/280 was determined to assess the purity of the sample. If the ratio is 1.8-2.0, the absorption is due to nucleic acids (Table 1). The quantity of DNA was calculated by using the formula. The amount and quality of DNA in all the samples were listed here under

1-OD = 50 mg DNA and taking the dilution factor

DNA mg/ml = A260 × dilution factor

The DNA sample (2 ml) from each isolate was mixed with 2 ml of 1 per cent loading dye on 0.8 per cent agarose gel along with 50 bp marker (TrackltTM 50 bp Thermo Fisher Scientific).

Dilution of stock DNA

The dilution of DNA (Table 2) was done to negate the formation of smearing banding pattern of DNA. Required final concentration of DNA for PCR analysis – 50 ng µL^{-1}.

$$\left.\begin{matrix}\text{Volume of stock DNA} \\ \text{to be added } (\mu l)\end{matrix}\right\} = \frac{\text{Required final concentration of DNA}}{\text{Actual concentration of DNA}} \times \text{Final volume}$$

DNA dilutions for Fall armyworm samples from

Table 1 : Nano drop spectrophotometer readings on DNA quantity of Fall armyworm.

Area of sample collection and Population Code	Purity (Absorbance at 260/280 nm)	Quantity (ng μL^{-1})
Ramakuppam-FAW21CHI	2.15	924.4
C.K. Dinne -FAW21KDP	1.73	2831.6
Nandikotkur- FAW21KRNL	2.07	1745.9
Chilmattur- FAW21ATP	2.08	1242.1
ARS (Agricultural research station), Perumalpalli- FAW21CHIRG	1.98	989.4

Table 2 : DNA dilutions for Fall armyworm samples from different geographic locations.

Area of samplecollection and Population Code	Stock DNA (µL)	Sterile water (µL)	Final volume of diluted DNA(µL)
Ramakuppam- FAW21CHI	5.36	94.64	100
C.K. Dinne- FAW21KDP	1.78	98.22	100
Nandikotkur- FAW21KRNL	2.86	97.14	100
Chilmattur- FAW21ATP	4.02	95.98	100
ARS Perumalpalli-FAW21CHIRG	3.01	96.99	100

Table 3 : List of primers used for PCR analysis and their sequences.

S. no.	Primer	Primer Nucleotide sequence	Primer length
1	LCO 1490	GGTCAACAAATCATAAAGATATTGG	25
2	HCO 2198	TAAACTTCAGGGTGACCAAAAAAT	24
3	OPA 01	CAGGCCCTTC	10
4	OPA 07	GAAACGGGTG	10
5	OPA 08	GTGACGTAGG	10
6	OPA 13	CAGCACCCAC	10
7	OPA 14	TCTGTGCTGG	10
8	OPC 08	TGGACCGGTG	10
9	OPC 15	GACGGATCAG	10
10	OPE 01	CCCAAGGTCC	10
11	OPE 02	GGTGCGGGAA	10
12	OPE 08	TCACCACGGT	10
13	OPE 15	ACGCACAACC	10
14	OPM 01	GTTGGTGGCT	10
15	OPR 02	CACAGCTGCC	10

different geographic locations were made by taking the stock solution obtained and made up the final volume to 100 (µL) by adding the remaining (µL) of sterile water.

Standardization of PCR

PCR conditions including temperatures, running durations and number of cycles for each step were optimized by altering them until good amplified product was obtained.

The primers (Table 3) were diluted from their original concentration of 100 pico moles to the working concentration of 10 pico moles using sterile distilled water.

PCR tubes of 0.2 ml were taken and 2 µL of DNA (100 ng μL^{-1}) was added. PCR reaction (Table 4) was performed in a 25 µL volume of mix.

Agarose gel electrophoresis

Agarose gel electrophoresis of PCR amplified DNA was performed as described by Sambrook and Russell (2001) in 1 per cent agarose gel (w/v) The gel was prepared by dissolving 1.0 g of MB (Molecular biology) grade agarose (Sigma, USA) in 100 ml of 1x TBE (Tris- borate- Ethylenediamine tetraacetic acid) buffer. The conical flasks along with its contents were placed in oven until agarose gel melted completely and clear solution was formed. Then the flask was taken out from the oven and allowed the solution to cool until it reached 50-55°C. Four micro litre of ethidium bromide (10 mg ml^{-1}) was added to 100 ml of agarose gel and mixed thoroughly. Later this solution was poured into the gel casting tray which is pre-set with 0.5 mm combs, slowly to avoid the formation of bubbles. After solidification, the comb was removed gently from the gel and then placed the gel with a casting plate in the gel tank. The DNA samples were mixed with 3 µL of 6X bromophenol blue loading dye (Fermentas, USA) and the electrophoresis was carried out in 1X TBE buffer at 100V (Labemate Power Pack 300, USA) till the dye front reached the lower part of the agarose gel. The migration pattern of the DNA fragments in the gel was recorded using gel documentation system (Alpha Innotech, USA) in an auto exposure mode.

Table 4 : PCR reaction mixture used for amplification of Fall armyworm DNA samples.

S. no.	Reagents	Concentration	Volume/ Tube
1	Taq assay buffer	10 X	2.5 µL
2	Magnesium chloride	25 mM	2.0 µL
3	dNTP's mix	100 mM	0.5 µL
4	Primer	10 p mol	2.0 µL
5	Taq polymerase enzyme	(3 u/ µL)	0.2 µL
6	DNA sample	100 ng/ µL	2.0 µL
7	Sterile distilled water	-	15.8 µL
	Total	-	**25.0 µL**

Scoring and data analysis for RAPD

Data was entered using a matrix in which all observed bands or characters were listed. The RAPD pattern of each population was evaluated by assigning character state 1 to all the bands that could be reproducible and detected in the gel and '0' for the absence of the band.

Results and Discussion

Molecular characterization using RAPD markers

In the present study, molecular characterization of total five populations collected during roving survey was undertaken using 13 RAPD markers. Among the 13 markers studied 5 showed polymorphism. The number of polymorphic bands indicates richness of the experimental material. Out of the 13 RAPD primers, 5 primers showed amplification and were polymorphic, they were OPA-07, OPE-01, OPE-02, OPC-15 and OPM-1 whereas eight primers (OPR-02, OPE-15, OPE-08, OPC-08, OPA-14, OPA-13, OPA-08, OPA-01) did not show any amplification. This may be due to insufficient attachment sites of the primer on template DNA. The total number of bands observed for every primer was recorded separately and polymorphic bands were checked subsequently. The total number of amplified bands varied between 7 (OPE-01) to 10 (OPA-07). Out of five primers, the number of RAPD loci generated was higher for the primer OPA-07 (10 loci) followed by primers OPC-15 (9 loci), OPE-02 (9 loci), OPM-01 (8 loci). The lowest number of loci were generated by the primer OPE-01 (7 loci). The overall size of PCR amplified products ranged between 100 bp to 1500 bp. The primer OPA-07 produced a total of 10 bands out of which 10 bands were found polymorphic similarly the primer OPE-01 produced a total of 7 bands out of which 7 bands were found polymorphic. Primers OPE- 02, OPC-15 and OPM-01 produced total bands of 9, 9 and 8 bands respectively, out of which 6, 7 and 6 bands respectively were polymorphic bands. The polymorphism is 100 per cent which is the highest for OPA -07, OPC- 15 followed by OPE-02, OPM-01 and OPE-01 with 77.77, 75.00 and 66.66 per cent, respectively (Fig. 1).

PIC value reflects the allele frequency and also acts as measure of informativeness of the markers and diversity among accessions. The PIC value of five polymorphic markers identified in the present study ranged from 0.27 (OPM-01) to 0.38 (OPA- 07). As per the classification given by Anh *et al.* (2018) markers showing PIC values between >0.25 and <0.5 were considered low informative markers. In the present study, markers with 100 per cent polymorphism had recorded PIC value between >0.25 and <0.5 and hence, markers used in the present investigation are classified as low informative markers.

DNA banding profile of individual Fall armyworm DNA samples from total five populations after amplification with primers is depicted in Fig. 1. The results of the experiment on molecular variability of Fall armyworm populations are in agreement with the results of Monnerat *et al*. (2006), who reported that the genetic similarity among the Fall armyworm populations from different geographic regions was in the range from 22 to 31 per cent. The results are also in confirmation with the findings of Martinelli *et al*. (2007), who found that the molecular similarity between FAW populations from different geographic locations varied from 14 to 72 per cent.

Janarthanan *et al*. (2002) found genetic difference between *S. litura* populations collected from six cotton growing fields of Tamil Nadu, using 40 RAPD markers and showed polymorphism. Gandhi and Patil (2016) demonstrated the value of RAPD markers for assessing the genetic variability between *S. litura* populations wherein a total of 3 random primers (OPA01, OPA-10, OPM-10) were screened to reveal the existence of polymorphism. In the present study, RAPD polymorphism is analyzed with a phenetic distance measure (Jacquard's coefficient) and dendrogram constructed to indicate diversity. The computation of similarity values was based on the presence or absence of discrete characters (PCR fragments) indicating number of PCR fragments shared (or not shared) between two individuals.

Genetic relationship between populations of Fall armyworm

Data of RAPD markers scanned from the 5 populations with 5 reproducible primers (Fig. 1) was used to generate similarity co-efficient. Jaccards similarity co-

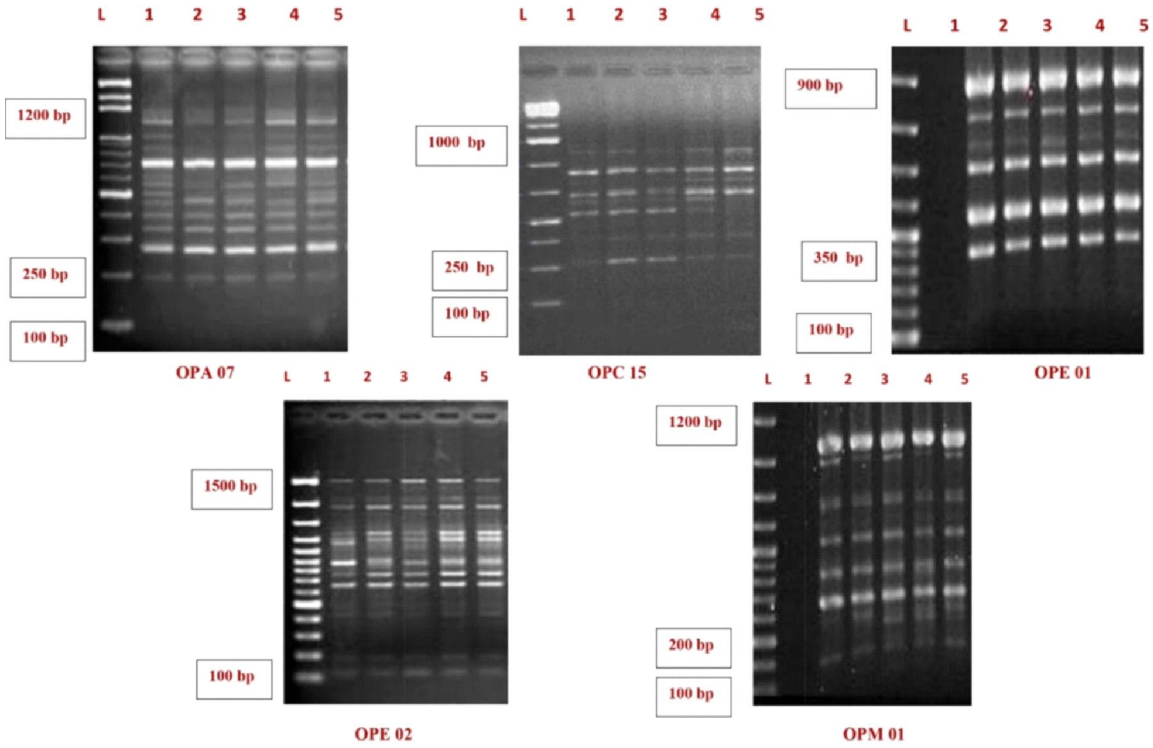

Fig. 1 : RAPD profile of 5 Fall armyworm larval populations using OPA 07, OPC 15, OPE 01, OPE 02 and OPM 01 Primers. **L = Ladder (100 bp), 1** = FAW21CH**, 2** = FAW21KRNL**, 3** = FAW21KDP**, 4** = FAW21ATP**, 5** = FAW21CHIRG.

Table -5: Jaccard's similarity co-efficient within populations of Fall armyworm. Proximity Matrix and Kruskal Wallis chi squared test

Case	Matrix File Input				
	FAW21CHIRG	**FAW21KDP**	**FAW21KRNL**	**FAW21ATP**	**FAW21CHI**
FAW21CHIRG	1.000				
FAW21KDP	.105	1.000			
FAW21KRNL	.267	**.269**	1.000		
FAW21ATP	.273	**.394**	.313	1.000	
FAW21CHI	.121	.242	.233	.281	1.000
Kruskal Wallis chi-squared	Chi square		4.000		
	df		4.000		
	P value		0.406		

efficient between the isolates is presented in Table 5. Similarity matrix thus produced indicated that the maximum genetic similarity value of 0.394 (39% similarity) was observed between Fall armyworm population on Maize in Ananthapuramu (FAW21ATP) and Kadapa (FAW21KDP) populations, while the lowest genetic similarity value of 0.105 (10% similarity) was recorded between Kadapa (FAW21KDP) and Fall armyworm population on Ragi in Chittoor (FAW21CHIRG) (Table 4). The Kruskal wallis chi squared test showed that though the populations are recording genetic variations they are not significantly different and statistically at par with one another.

Phylogenetic analysis based on RAPD

The phylogenetic relationships among populations of Fall armyworm were analyzed by an UPGMA method (Fig. 2). The cluster tree analysis showed that populations were divided into two major clusters having populations of Kadapa, Kurnool and Ananthapuramu in one cluster, populations of Chittoor and Fall armyworm population on Ragi in Chittoor into another cluster.

$$\text{Per cent polymorphism} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

Cluster analysis was performed by using the DARWIN version 6.0.010 software. Jaccard's similarity coefficient was calculated by using the data matrix for each pair-wise comparison. To group the genotypes based on their overall similarities, the similarity coefficients were subjected to an Unweighted Pair Group Method with Arithmetic averages (UPGMA). Our investigations about genetic variations in FAW reveal a strong variability between different samples. The genetic divergences observed between sites in *S. frugiperda* may possibly come from two main factors, *i.e.*, spatial pattern and pest management practice. In moths, differences in genetic variability were frequently observed in populations selected from different host plants *viz.*, Fall armyworm population collected on Maize segregated as one cluster and Fall armyworm population collected on Ragi was entirely separated into another cluster that did not fall under the clad of population belonging to Maize.

Specific host-plants greatly influences genetic diversification of insect species. From the above stated result, it is suggested that this variation might be due samples collected from different locations exhibit a high degree of genetic variability tending to the phenols and sugars variation in plants survey as well due to disparity in many factors such as host phenology variation in Maize and Ragi, climatatic variation among districts of Rayalseema, where in Heavy rainfall (>111.85 mm) was witness in the districts of Ananthapur and Kadapa during the periods of survey where the population was noticed to show a gradual decline in number due to the natural infestation of *Metarhizium rileyi* infested cavaders observed in fields, where as in case of Kurnool and chittoor districts, indiscrimate usage of Emamectin benzoate and Spinoteram was recorded in areas surveyed, in spite of using the chemicals, pest was prevalent indicating a probable development of pesticide resistance in the population.

Fluctuating temperatures (28.7-42 °C) and Erratic rainfall ranging from (99.2-111.85mm) recorded during study would have created tremendous selection pressure for survival responsible for the diversity of insect population. Hence, there might be chances of variablity in the gene pool due to adoption and survival of the insect population in diverse environmental conditions. The present results are in accordance with studies conducted on *S. litura* having high dispersal rate, resulting into no boundary or geographical limitations effect (Saito, 2000). Tremendous selection pressure of insectisides might also create the gene level alteration of the populations of *S. litura* (Karuppaiah *et al*., 2017). Chandola *et al*. (2011) conducted studies in upper hills of Uttarakhand and

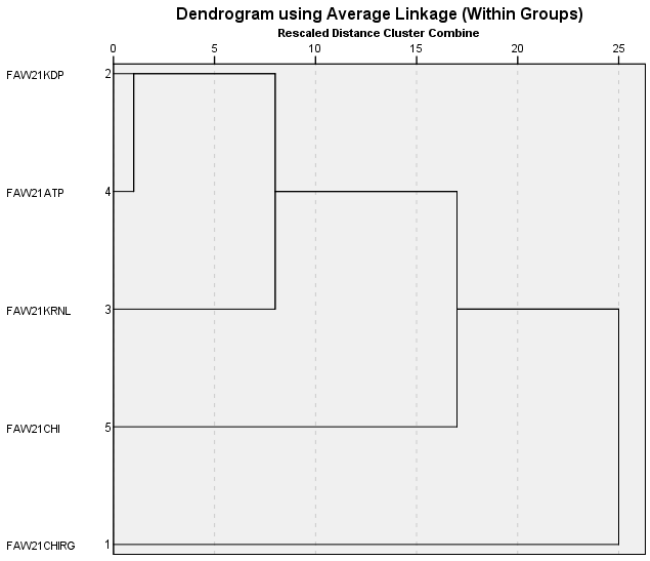

Fig. 2 : Dendrogram depicting variation among populations of Fall armyworm based on RAPD analysis.

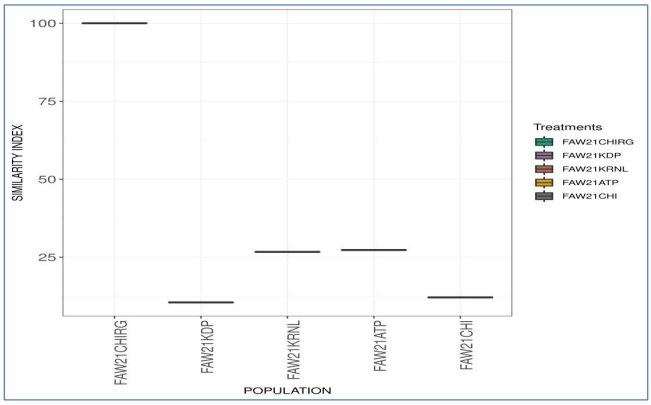

Fig. 3 : Box plot depicting variations among populations of Fall armyworm.

confirmed that farmers always tend to use the indigenous technology for controlling the pest population. In plains due to use of pesticides, population undergoes tremendous selection which ultimately makes the alteration in the gene level.

Interestingly, the results of our population-genetic study in *S. frugiperda* through. RAPD and statistical analyses showed that highly-resistant population (Chittoor and Kurnool *i.e.*, FAW21CHI, FAW21CHIRAG and FAW21KRNL) clearly diverge (Fig. 3) genetically from low-resistant populations (FAW21ATP, FAW21KDP). Analyzing RAPD banding pattern, genetic matrix, Jaccard similarity index these populations fall into two distinct clusters. In addition to having difference in resistance capacity, they do not have the same genetic profile despite their close geographical distances. In moths, subtle genetic changes have been shown to lead variations in reproductive traits such as pheromone production within different species.

Conclusion

S. frugiperda is having high dispersal rate and tremendous selection pressure of insectisides could also have created the gene level alteration. Hence, RAPD analysis and Jaccords similarity index matrix help to segregate the population into clads which gives us a clear picture of taking up further studies such as Rice strain and Corn strain variation among emerging Fall armyworm population. Since this is the beginning of spread of the insect, Kruskal wallis test from the present investigation proved that there are no significant differences among the genetic variations statistically, it is a forewarning alarm that due to this extensive spreading nature, if proper management strategies are not devised there is every chance that these genes will show significant variations within the population.

Acknowledgements

My deep sense of gratitude is to S. V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University for providing the required facilities to conduct the experiment.

References

Bharathiraja, C., Sukirtha R., Anbalagan S., Soundrapandian K., Meenakshi S., Krishnan M. and Jean F.P. (2013). Intra Populational Genetic Diversity In The Tobacco Armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Asian J Biochemic Pharma Res*., **3(2)**, 100-113.

Chandola, M., Rathore S. and Kumar B. (2011). Indigenous pest management practices prevalent among hill farmers of Uttarakhand. *Ind J Trad Knowl.,* **10(2)**, 311-315.

Davis, F.M .and Williams W.P. (1992). Visual rating scales for screening whorl-stage corn for resistance to fall armyworm. *Mississippi Agricultural and Forestry Experiment Station, Technical Bulletin 1 Mississippi State University*, MS39762, USA. 29-34.

Gandhi, B.K. and Patil R.H. (2017). Genetic diversity in *Spodoptera litura* (Fab.) from major soybean growing states of India. *Leg Res*., **40(6)**, 1119-1125.

Ganiger, P.C., Yeshwanth H.M., Muralimohan K., Vinay N., Kumar A.R.V. and Chandrashekara K. (2018). Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), in the maize fields of Karnataka, India. *Curr Sci.,* **115(4)**, 621-623.

Ginting, S., Agustin Z., Risky H.W. and Sipriyadi S. (2020). New invasive pest, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) attacking corns in Bengkulu, Indonesia. *Serangga*, **25(1)**, 105-117.

Goergen, G., Kumar P.L., Sankung S.B., Togola A. and Tamò M. (2016). First Report of Outbreaks of the Fall Armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a New Alien Invasive Pest in West and Central Africa. *PLOS ONE*, **11(10)**, 19-26.

Jain, S.K., Neekhra B., Pandey D. and Jain K. (2010). RAPD marker system in insect study: A review. *Indian J Biotech*., **9(2)**, 7-12.

Janarthanan, S., Seshadri S., Kathiravan K. and Ignacimuthu S. (2002). Use of RAPD in assessing the genetic variability in *Spodoptera litura* Fab. *Indian J Exp. Biol*., **40**, 839-841.

Kalleshwaraswamy, C.M., Asokan R., Swamy H.M., Maruthi M.S., Pavithra H.B., Hegde K., Navi S., Prabhu S.T. and Goergen G. (2022). First report of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Mangt Hort. Ecosys*., **24(1)**, 23-29.

Karuppaiah, V., Srivastava C. and Subramanian S. (2017). Toxicity and effectiveness of newer insecticides, conventional insecticides mixtures to field populations of *Spodoptera litura* (Noctuidae: Lepidoptera). *J Entomol Zool. Stud*., **5(6)**, 1893-1897.

Martinelli, S., Clark P.L., Zucchiz M.I., Silva-Filho M.C., Foster J.E and Omotozz C. (2007). Genetic structure and molecular variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) collected in maize and cotton ûelds in Brazil. *Bull. Entomol Res.*, **97(1)**, 225-231.

Mritunjoy, B., Neeta G., Rashmi J., Rukesh P. and Pradeep M. (2019). Rapd- analysis of populations of *Spodoptera litura* (f.) from Uttarakhand. *Indian J Entomol*., **81(4)**, 841-846.

Murray, M.G. and Thampson W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucl. Acids Res*., **8(1)**, 4321- 4325.

Saito, O. (2000). Flight activity of three *Spodoptera spp.*, *Spodoptera litura, S. exigua* and *S. depravata*, measured by flight actograph. *Physiol. Entomol.*, **25(2)**, 112–119.

Sharanabasappa, Kalleshwaraswamy C.M., Asokan R., Mahadevaswamy H.M., Maruthi M.S., Pavithro H.B., Hegde K., Navi S., Prabhu S.T. and Goergin G. (2018). First report of fall army worm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) an alien invasive pest on maize in India. *Pest Mangt Hort Ecosys.*, **24(1)**, 23-29.

Visalakshi, M., Ashika T.R., Venkatesan T., Ballal C.R., Laxman K., Nagarjuna D., Chittibabu G., Venkatarao P. and Jamuna P. (2019). Report of the invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and its natural enemies on maize and other crops from Andhra Pradesh, India. *J Entomol Zool Stud*., **7(4)**, 1348-1352.

Williams, J., Anne R. Kubelik, Livak J.K., Rafalski J.A. and Tingey S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids*, **18(22)**, 6531–6535.