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# Genetic Variability of Tobacco Caterpillar *Spodoptera litura* (F.) Infesting Groundnut

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***Abstract*:** The genetic variability of *Spodoptera litura* populations in Andhra Pradesh was assessed during 2017–18 at RARS, Lam with populations collected from Guntur, Prakasam, Krishna, Vizianagaram, and Chittoor districts. DNA extracted from larvae using the HiMedia DNA kit was amplified with RAPD markers. Of the 12 primers tested, four primers (OPA-3, OPA-4, OPC-5, and OPC-9) successfully amplified all populations. Total of 33 bands were generated, showing 65.9% polymorphism. Jaccard similarity coefficients ranged from 0.61–1.00. The lowest similarity was observed between the Guntur and Vizianagaram populations and between Chittoor and Vizianagaram populations. The highest similarity was between Prakasam and Vizianagaram, and between Krishna and Chittoor populations.

***Keywords*:** *Spodoptera litura,* Genetic variability, Polymorphism, Jaccard similarity coefficients

Tobacco caterpillar, *Spodoptera litura* Fabricus (Lepidoptera: Noctuidae) has become an economic important pest of tobacco, cotton, rice, maize, soybean and groundnut over the years. And caused economic crop losses from 25.8 to 100 per cent depending on the stage of the crop and its intensity in the field (Natikar and Balikai 2015). Synthetic pyrethroids were introduced in 1982 for the management of tobacco caterpillar on cotton in Andhra Pradesh in response to rising control failures due to insecticide resistance (Rao et al., 2007). By the late 1980s, the pest had become widely regarded as uncontrollable by farmers and research–extension personnel, Mehrotra (1997) reported that *S . litura* had already developed multiple resistance, particularly to pyrethroids. Subsequent studies further established widespread resistance to several insecticide groups. Armes et al. (1997) documented resistance cypermethrin, fenvalerate, endosulfan, quinalphos, monocrotophos, and methomyl. Kranthi et al. (2002) also reported high resistance levels during 1997–98 to commonly used insecticides such as chlorpyrifos in *S. litura* populations from Warangal, Medak and Amaravathi. More recently, resistance to novel insecticides including abamectin, emamectin benzoate, fipronil, indoxacarb, spinosad, and chlorantraniliprole has been reported in several countries (Ahmad et al., 2008, Shad et al., 2010, Shad et al., 2012, Su et al., 2012, Tong et al., 2013).

Further, the issue involved is whether all *S. litura* populations across Andhra Pradesh exhibit high insecticide resistance or whether resistance is localized remains unclear. Genetic variability among geographically distinct populations often contributes to differences in insecticide resistance potential. Understanding such variability is essential for designing effective surveillance programs and location-specific IPM strategies. Variations in genetic structure and the molecular characterization of a pest population in space and time, the gene flow among sub populations are incredibly accountable for the rate of resistance evolution (Fuentes-Contreras et al., 2004). Therefore, molecular markers have been extensively used to evaluate genetic similarity and estimate gene flow among insect populations. The Random Amplification of Polymorphic DNA (RAPD) method described by Williams et al. (1990) produces PCR products by annealing to homologous target sites randomly distributed the template DNA. Thus, RAPD markers are traditionally used to investigate genetic similarity and population structure as described for many different insect species (Zhang et al., 2005). Using RAPD and later Amplified Fragment Length Polymorphisms (AFLP) protocols, different populations of *Spodoptera frugiperda* (Smith) have been successfully genetically characterized (Martinelli et al., 2006). Considering the importance of *S. litura* as a major polyphagous pest, understanding its genetic diversity across major crop-growing regions is crucial for anticipating resistance evolution and designing location specific sustainable pest management strategies.

## MATERIAL AND METHODS

Genetic variability of *Spodoptera litura* in Andhra Pradesh was conducted during 2017-18 at RARS, Lam

**DNA extraction:** Larvae of *S. litura* were collected from groundnut fields in six different districts of Andhra Pradesh *viz*., Vizianagaram 18.10649, 83.14903, Krishna 16.22853, 81.07406, Guntur 15.90732, 80.50671, Prakasam 15.7891,

79.664762, Kurnool 15.593205, 77.570829 and Chittoor 13.694836, 79.589327 (Fig. 1). The larval population was collected in sterile plastic vials poured with 70 percent ethanol and brought to the Entomology Laboratory, Regional Agricultural Research Station, Lam, and stored at -20 $^{O}$C until DNA extraction. The DNA extraction was done using Himedia kit. *S. litura* collected from six districts of Andhra Pradesh were weighed (not more than 30 mg) and grounded using mortar and pestle in liquid nitrogen to a fine powder. The tissue powder was transferred to a clean capped 2.0 ml microcentrifuge tube. The DNA was quantified using a Nanodrop spectrophotometer at 260nm. Twelve random primers (Operon technology) *viz.,* OPA-1, OPA-2, OPA-3, OPA-4, OPA-5, OPA-9, OPA-11, OPA-13, OPA-20, OPC-2, OPC-5, and OPC-9 were screened. Four primers with high polymorphism, *i.e*., OPA-3, OPA-4, OPC-5, and OPC-9 were used for generating polymorphism among the *S. litura* populations. The experiment was repeated thrice, and the results were reproducible.

PCR amplification was performed using random primers in the Eppendorf master cycle. The PCR conditions were optimized in the concentration of template DNA from 50 ng to 100 ng in a reaction volume of 25 µl. A reaction volume of 25 µl and 100 ng of DNA gave a maximum number of reproducible bands and thus was considered ideal and used subsequently in the analysis.

**Agarose gel electrophoresis:** Amplified products were separated on 1.5 per cent agarose gel stained with ethidium bromide (0.5µg/ml of gel). Agarose gel 1.5 percent (w/v) was prepared by dissolving 1.5 g of agarose in 100 ml of 1x TBE buffer. The gel was allowed to cool for some time, and then two µl of ethidium bromide (10 mg/ml) was added. 3µl of loading dye was added to 25 µl of PCR product and mixed well before loading into wells (Appendix). Electrophoresis was conducted at 100 volts for 2 hours, and the gel was photographed under U.V. light using gel doc system (Alpha Innotech, USA). The PCR Amplification conditions were: initial denaturation at 94$^{0}$C for 5 minutes, 40 cycles of Denaturation at 94°C for 1 minute, Annealing at 38°C for 1-minute Elongation at 72°C for 2 minutes each and Final elongation at 72°C for 7 minutes.

**Similarity coefficient:** Jaccard's similarity coefficient was calculated by using the data matrix. The Similarity coefficient (Jaccard, 1908) index was deduced 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.

$$\text{Percent polymorphism} = \frac{\text{Total no. of polymorphic bands}}{\text{Total no. of bands}} \times 100$$

The similarity coefficients were subjected to the Unweighted Pair-Group Method of Arithmetic averages (UPGMA) cluster analysis for grouping the genotypes based on their overall similarities using NTSYspc-2.02i software (Rohlf 1998)

**Statistical analysis:** Data entry was done in a binary matrix where all observed bands were listed-assigning character states '1' for those where the bands were reproducible and '0' for those where the bands were absent in the RAPD pattern for each genotype.

## RESULTS AND DISCUSSION

**RAPD profiling for different populations of *S. litura:*** Of the 12 primers evaluated, four primers consistently amplified DNA from all six *S. litura* populations. These primers

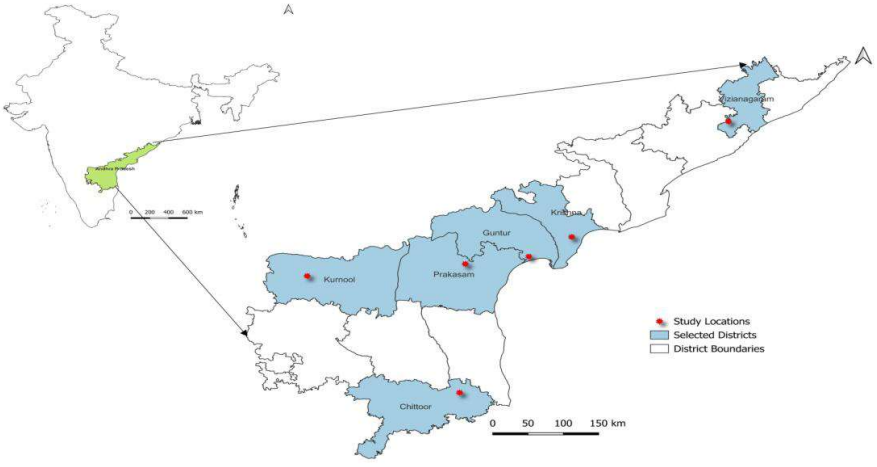


**Fig 1.** Map showing study area with GPS locations

produced 33 total bands, of which 65.9% were polymorphic (Fig. 2). OPA-3 produced nine bands that were 66.6% polymorphic, and the band size ranged between 250-2000 bp. OPA-4 had given eight polymorphic bands that varied from 300-3000 bp. OPC-5 generated nine polymorphic bands that ranged between 300-3000bp with 77.7% polymorphism. OPC-9 produced seven polymorphic bands of 200-5000 bp band size with 57.1% polymorphism (Table 1).

**Jaccard similarity coefficients**: The Jaccard similarity coefficients among the six *S. litura* populations varied between 0.61 to 1.00. The lowest similarity of 0.61 was observed between Guntur and Vizianagaram, Chittoor and Vizianagaram populations, while the highest similarity was noticed between the populations of Prakasam and Vizianagaram, Krishna and Chittoor at 0.85 (Table 2).

**Dendrogram Analysis for the different populations of *S. litura*:** UPGMA cluster analysis grouped the six *S. litura* populations of Andhra Pradesh into two major clusters (Fig. 3). Of the two main clusters, four populations formed one big cluster, and two remained as another small cluster. The Vizianagaram population formed a close cluster with Prakasam, sharing 85% similarity. The second major cluster comprised the Krishna and Chittoor populations, which also

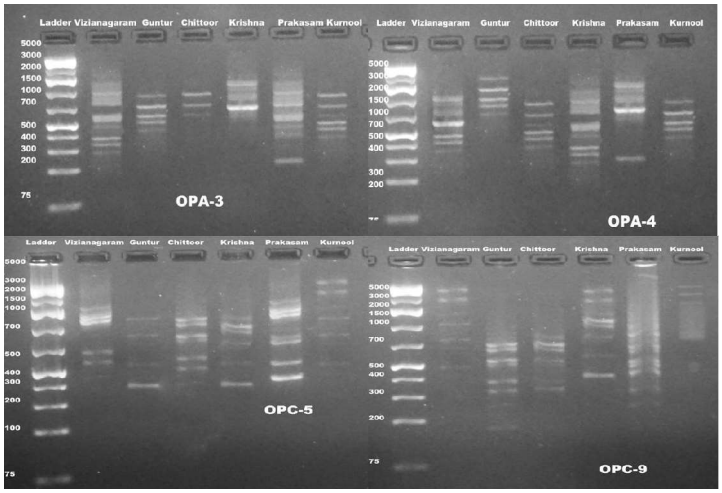


**Fig. 2.** RAPD profiling for different populations of *S. litura* with OPA-3, OPA-4, OPC-5, and OPC-9 primers

**Table 1.** Percent polymorphism in *S. litura*

| Primer | Sequence | Total no. of bands | Polymorphic bands | Polymorphism (%) | Band size (bp) |
|---|---|---|---|---|---|
| OPA-3 | 5'AGTCAGCCAC3' | 9 | 6 | 66.6 | 250-2000 |
| OPA-4 | 5'AATCGGGCTG3' | 8 | 5 | 62.5 | 300-3000 |
| OPC-5 | 5'GATGACCGCC3' | 9 | 7 | 77.7 | 300-3000 |
| OPC-9 | 5'CTCACCGTCC3' | 7 | 4 | 57.1 | 200-5000 |
| Total | | 33 | 22 | 65.9 | |

**Table 2.** Jaccard similarity coefficients between different populations of S. *litura*

| | Vizianagaram | Guntur | Chitturu | Krishna | Prakasam | Kurnool |
|---|---|---|---|---|---|---|
| Vizianagaram | 1 | | | | | |
| Guntur | 0.61 | 1 | | | | |
| Chitturu | 0.61 | 0.76 | 1 | | | |
| Krishna | 0.64 | 0.79 | 0.85 | 1 | | |
| Prakasam | 0.85 | 0.64 | 0.64 | 0.67 | 1 | |
| Kurnool | 0.69 | 0.73 | 0.73 | 0.76 | 0.67 | 1 |

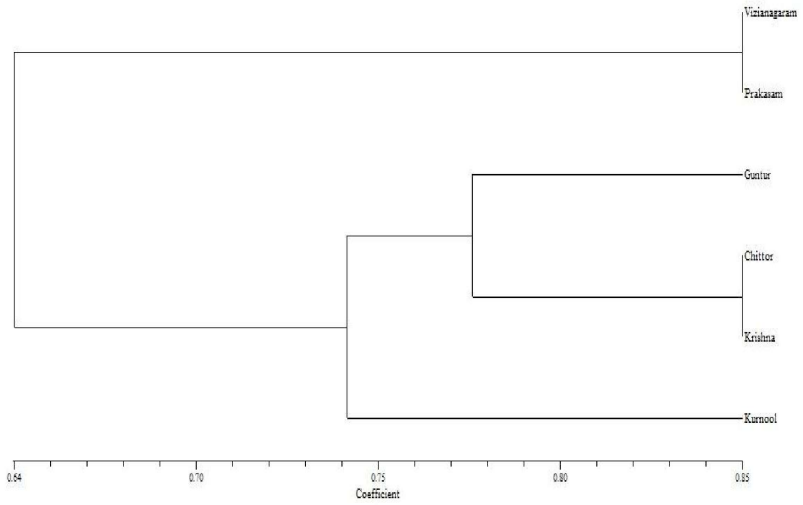


**Fig. 3.** Dendrogram for different populations of *S. litura*

exhibited 85% similarity and together showed 78% similarity with the Guntur population. Among all populations, Kurnool was the most genetically distinct, displaying 74% dissimilarity from the Guntur–Chittoor–Krishna group.

The present studies are in accordance with Barman (2019) indicating pronounced genetic variability among *S. litura* populations in the Kumaon region, noting that the strain collected from the plains showed only 32 per cent similarity with strains from the mid-hills, valley, and Tarai regions, indicating substantial genetic divergence. Such variability suggests that new biotypes with enhanced insecticide resistance may emerge from this diverse gene pool. Similarly, Gandhi and Patil (2017) reported considerable variation among seven *S. litura* populations from soybean fields, with genetic similarity ranging from 46 to 100 per cent. Hyderabad and Indore populations shared complete similarity, while Pune and Parbhani recorded 90 per cent similarity, populations from Adilabad, Hyderabad, and Indore showed 83 per cent similarity, and Dharwad and Belagavi exhibited 72 per cent similarity. The lowest similarity (46%) was observed between the Dharwad and Parbhani populations, Janarthanan et al. (2002) also assessed genetic variability in six ecotypes of *S. litura* using RAPD markers. Of the 40 random primers screened, only three (OPA-1, OPA-5, and OPM-1) produced clear and distinguishable banding patterns. Their analysis showed that the Chengalpattu and Chennai populations were genetically closer to each other, whereas the Coimbatore population was more distant from the remaining ecotypes. Similarly, Bharathiraja et al. (2013) reported high genetic variability among *S. litura* populations collected from ten locations across Tamil Nadu, with RAPD polymorphism ranging from 90–100%, indicating strong genetic differentiation. Gandhi and Patil (2017) observed high similarity (90%) between Pune and Parbhani *S. litura* populations, while the lowest similarity (46%) was recorded between Dharwad and Parbhani populations. Bharathiraja et al. (2013) demonstrated the highest polymorphism of 90 to 100% in RAPD analysis, confirm the presence of strong genetic polymorphism among geographically distinct *S. litura* populations collected from different castor fields in South India. The observed genetic variability in the present study indicates substantial genetic structuring among *S. litura* populations in Andhra Pradesh. Geographical and chronological factors might have influenced the presence of prevailing diversity among the populations. Such variability can facilitate the emergence of new biotypes with different insecticide resistance potentials, emphasizing the need for region-specific IPM strategies.

## CONCLUSION

The substantial genetic variability among *S. litura* populations collected from different agro-ecological regions, reflecting the dynamic nature of this polyphagous pest. UPGMA clustering clearly separated the populations into distinct groups, indicating region-specific genetic structuring and possible adaptation to local host plants and environmental conditions. These results emphasize the need for region-specific management strategies and continuous monitoring of population structure to design effective, sustainable pest management programs.

## AUTHOR'S CONTRIBUTION

Conceptualization and experiment design: Dr. GMV Prasada Rao, Trial execution and data collection: Ms. Annie Diana Grace, Data curation, statistical analysis and draft preparation: G. Annie Diana Grace, Dr. GMV Prasada Rao.

Dr. PV Krishnayya, Dr. V. Manoj Kumar. And Dr V. Srinivasa Rao Final draft revision and approval: G. , Annie Diana Dr. GMV Prasada Rao. Dr. PV Krishnayya, Dr. V. Manoj Kumar. And Dr V. Srinivasa Rao. The authors declare no competing interest exists.

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