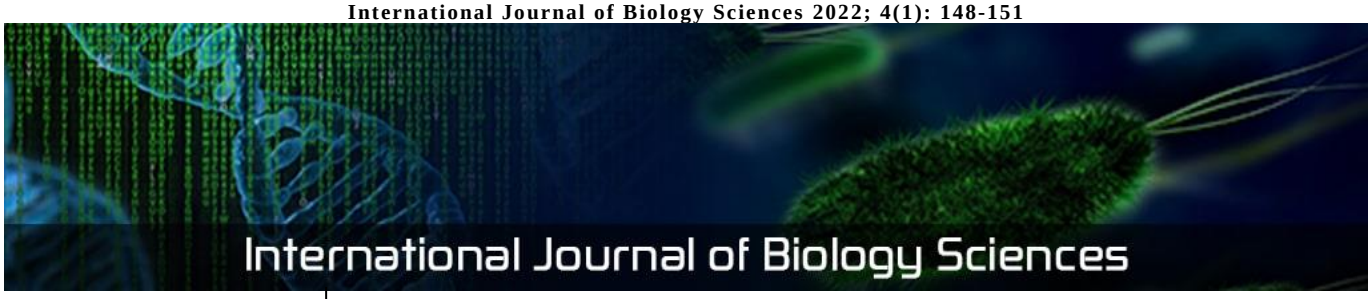

ISSN Print: 2664-9926
ISSN Online: 2664-9934
Impact Factor: RJIF 5.45
IJBS 2022; 4(1): 148-151
www.biologyjournal.net
Received: 09-04-2022
Accepted: 15-05-2022

Karthik V Rao
Etho-Biomolecules Laboratory, Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Manjulakumari Doddamane
Etho-Biomolecules Laboratory, Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Corresponding Author:
Manjulakumari Doddamane
Etho-Biomolecules Laboratory, Department of Microbiology and Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore, Karnataka, India

Molecular analysis of *Spodoptera frugiperda* from India using 28S nuclear genes markers

Karthik V Rao and Manjulakumari Doddamane

DOI: https://doi.org/10.33545/26649926.2022.v4.i1d.129

Abstract
The fall army worm *Spodoptera frugiperda* (J E Smith), commonly called as fall armyworm (FAW) (Lepidoptera: Noctuidae) is a highly voracious insect pest which is native to the tropical and subtropical regions of America. It is one of the most serious pests of maize in India. In this study, we have collected mainly larvae of *S. frugiperda* from the maize fields of across India using 28S nuclear gene as markers. The larvae exhibited sexual dimorphism in their morphological characteristics. BLAST search on Indian specimen's revealed 98-99% nucleotide sequence identity with S. FAW voucher specimens from France and Western South America of either maize or rice populations. The phylogenetic relationship was inferred using IQ Tree1.6.12 built with input data of 54 sequences with 867 nucleic acid sites and using the best model BIC: F81+F Model with ultrafast bootstrap (1000 replicates) support. Phylogeny tree generated from the *Spodoptera frugiperda* of 28S data from this study alone did not show any major clades or differences. The present study revealed that the Indian maize populations were clustered in clade one which had a high degree of geographical representation consisting of Guadeloupe (France), and Peru (Western South America) maize/rice populations. As this pest is reported to have remarkable dispersal capacity, high reproductive capacity, absence of diapause, and wide host range, it is likely to spread to the entire country and further, neighbouring countries.

Keywords: Fall armyworm, maize, morphological characters, phylogenetic relationship, dispersal capacity

Introduction
Spodoptera frugiperda (JE Smith), commonly called as fall army worm (FAW) (Lepidoptera: Noctuidae) is a highly voracious insect pest which is native to the tropical and subtropical regions of America. It has a wide host range of >353 host plants (Montezano *et al*., 2018) [9]. The most frequently fed and damaged plants are maize, sorghum, rice, millet, soybean, peanut, cotton, Sudan grass, and other fodder grasses. Among different host plants, maize was the major crop infested. In India, FAW was first reported on maize from Shivamogga district of Karnataka (Sharanabasappa *et al*., 2018). It is highly migratory and has rapidly spread all over the country and reported in neighbouring countries like Nepal and China (Rathna *et al*., 2019; FAO 2019) [12]. The incidence on maize ranged from 9.0 to 62.5% and 6 to 100% (Shylesha *et al*., 2018; Mallapur *et al*., 2018) [16, 7] in different districts of Karnataka. There are two strains that are morphologically indistinguishable but differ in their host plant preference. The Rice (R) strain most consistently feeds on rice, Bermuda grass, and other small grasses, while the Corn (C) strain prefers maize, sorghum, and other large grasses (Pashley *et al*., 1985). Recent studies on its population in India have revealed that the majority of these are 'R' strain feeding on maize, sorghum and sweet corn; while 'C' strain feeds on sugarcane (Mahadevaswamy *et al*., 2018; Chormule *et al*., 2019) [2]. The knowledge on its strains and genetic diversity is important for strategizing pest management. (Srinivasan *et al*., 2018) [17]. Hence, in this study we have explored the bio-geographical distribution pattern using 28S nuclear gene.

Materials and Methods
To know the status of FAW, larvae were collected from different geographical areas and reared in laboratory. While some were reared on artificial media (Shobha *et al*., 2009) [15], some were reared on natural host, i.e., maize leaves in individual boxes to see any difference in their development and growth. The abdomen/legs/thorax of larvae/adult were used to extract DNA by CTAB method for molecular analysis (Reineke *et al*., 1998) [13].

The extracts were subjected to PCR amplification at 867bp using primers specific to the region near the 5' terminus of the 28S gene from the genomic DNA using primers > 28S-01-5'GAC TAC CCC CTG AAT TTA AGC AT 3' and 28SR-01-5'GAC TCC TTG GTC CGT GTT TCA AG 3' <. PCR reactions were carried out in 96 wells PCR with 50µl reaction volume containing GeNei™ Taq buffer 5µl, 1µl of GeNei™ 10 ml dNTP mix, 2.5µl of (20 pmol/µl) forward primer, 2.5µl of (20 pmol/µl) reverse primer, 1 µl of GeNei™ Taq DNA polymerase (1 U/µl), DNA (50 ng/µl) 2µl, and sterile water 36µl. PCR was performed using a C1000™ Thermal Cycler of Biorad with Initial denaturation at 94 °C for 3 min, Cycle denaturation with 92 °C for 1 min, annealing temperature of 54 °C for 1 min, extension for 70° c for 30 sec, followed by final extension at 70 °C for 10 min and kept in 40c until use. The amplicon was visualised on 1.2% agarose gel containing 0.5 µg/ml of ethidium bromide and the amplified product was purified using Qiagen Mini elute PCR purification kit (Qiagen India), quantified using Nano drop1000 (Thermo fisher scientific, USA).

The purified fragments were sequenced by M/S Biokart India Pvt. Ltd., Bengaluru, India. Sequences were edited manually and aligned using Clustal W and submitted to NCBI GenBank. The sequences were verified and edited using BioEdit Sequence Alignment Editor Version 7.2.5 (Hall 1999). The sequences were aligned using MUSCLE (Edgar 2004) [3] in MEGA 11 (Tamura *et al*. 2021) [18]. IQ-TREE (Nguyen *et al*. 2015) [10] was used for performing the maximum likelihood method of phylogenetic inference. A preliminary phylogenetic analysis was run to identify any doubtful sequences and those were subsequently omitted from the analysis. The optimal models were determined through Model Finder (Kalyaanamoorthy *et al*. 2017) [6] in IQ-TREE, using the minimum BIC score. Statistical support for the clades in ML was assessed by Ultrafast bootstrap (BP) for 10,000 iterations, 0.1 perturbations, and 500 num stops (Minh *et al*. 2013) [8] using IQ-TREE web server (Trifinopoulos *et al*. 2016) [19]. The tree obtained from ML analyses was visualized using Fig Tree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree).

Results and Discussion

Morphological data

The fall army worm *Spodoptera frugiperda* under the family Noctuidae is one of the most serious pests of maize in India. In this study, we have collected mainly larvae of *S. frugipedra* from the maize fields of across India.

The eggs are dome shaped; the base of the egg is flattened and the egg gets curved upward with a broadly rounded apex. The freshly hatched larvae measured 1 mm in length and 0.5 mm in width (Fig 1). Pupae were of dark brown in colour. The pupal body measured about 17 mm and had a width of 5 mm. Adults emerged within 7-8 days after pupation (Fig 2).

Adult moths 32 to 40mm with a conspicuous spot on the forewings, which have a wavy patterns on the fringe. The insects measured about 15 mm in length and had a wing span of 30-35 mm. The adult moths exhibited sexual dimorphism in their morphological characteristics. Males were dark greyish with white markings on forewings. They possessed large tufts of hair on the forelegs. Females lacked both white markings and tufts of hairs (Fig 3). On the whole, the embryonic period lasted 2-3 days, larval period 19-23 days and pupal period 7-8 days and adults average about 10 days, with the range of about seven days to 21 days.

Fig 1: Larvae

Fig 2: Adult Moth

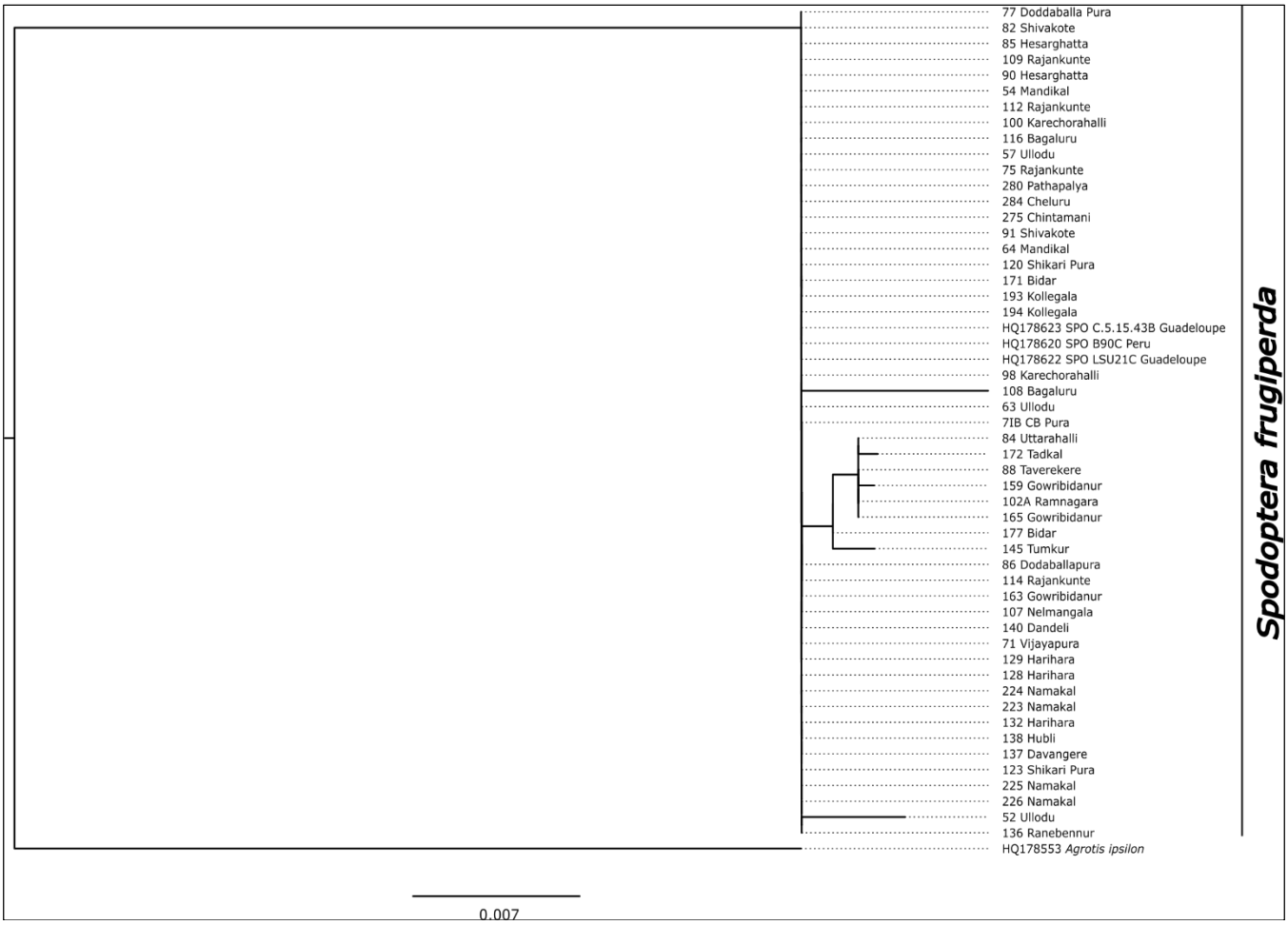

Fig 3: The Phylogenetic relationship of *Spodoptera frugiperda* samples from Maize populations of India, inferred from the 28S sequence (867bp) using the IQ-TREE based on Maximum Likelihood method. (NCBI Data of **Spodoptera frugiperda*, ***Agrotis epsinon* an out group

Sequence analysis

Fragments amplified from 28s primer pairs aligned to their target reference sequence. The search and analysed results indicated that they belong to *S. frugipedra*. The *S. frugipedra* 28S sequences from Indian population were identical to each other, and they were 87-100% identical compared to sequences from other countries. BLAST search of Indian specimen's revealed 98-99% nucleotide sequence identity with *S. frugipedra* voucher specimens from France and Western South America of either maize or rice populations. The base identification from BLAST search also shows that Indian fall armyworm belongs to *S. frugipedra* sps with 99.66% similarity.

S. frugipedra sequences from India are deposited with NCBI Gen Bank.

Phylogenetic Analysis

Phylogenetic tree generated from the *S. frugipedra* of 28S data from this study alone, did not show any major clades or differences. The present phylogenetic analysis consisted of fifty *Spodoptera. frugipedra* 28S sequences generated from this study and three from NCBI Gene bank. The 28S sequence of *Agrotis ipsilon (HQ178553)* accession number served as an out group for the analysis. The evolutionary relationship was inferred using IQ Tree1.6.12 built with input data of 54 sequences with 867 nucleotide sites and using the best model BIC: F81+F Model with ultrafast bootstrap (1000 replicates) support. The bootstrap frequencies converged at 0.991 after 9300 iterations. The tree generated by combining the NCBI Genbank sequences showed one major clade (FIG 1).The *S. frugipedra* Indian maize populations were clustered in clade 1.which had a high degree of geographical representation consisting of Guadeloupe (France), and Peru (Western South America) maize or rice populations. This indicates the species have migrated from France and Western South America to India.

At this point, the point of entry of the fall army worm into India is not yet determined but could not decipher based on the trade analysis and employing haplotype analysis. One of the possible modes of infestation is through agricultural commodities. As this pest is reported to have remarkable dispersal capacity, high reproductive capacity, absence of diapause, and wide host range, it is likely to spread to the entire country and further, neighbouring countries.

References

1. Chormule A, Shejawal N, Sharanabasappa, Kalleshwaraswamy CM, Asokan R, Mahadeva Swamy HM. First report of the fall armyworm, Spodoptera frugiperda (J.E. Smith) (Lepidoptera, Noctuidae) on sugarcane and other crops from Maharashtra, India. Journal of Entomology and Zoology Studies. 2019b;7(1):114-117.
2. Chormule A, Shejawal N, Sharanabasappa CM, Asokan R, Swamy HM, Studies Z. First report of the fall Armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae) on sugarcane and other crops

from Maharashtra, India. J Entomol. Zool. Stud. 2019;7(1):114-117.
3. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research. 2004;32(5):1792-1797.
4. Giribet G, Wheeler WC. On gaps. Molecular phylogenetics and evolution. 1999;13(1),132-143.
5. Kalleshwaraswamy CM, Maruthi MS, Pavithra HB. Biology of invasive fall army worm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) on maize. Indian journal of entomology. 2018;80(3):540-543.
6. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. Model Finder: fast model selection for accurate phylogenetic estimates. Nature methods. 2017;14(6):587-589.
7. Mallapur CP, Naik AK, Hagari S, Prabhu ST, Patil RK. Status of alien pest fall armyworm, Spodoptera frugiperda (JE Smith) on maize in Northern Karnataka. J Entomol. Zool. Stud. 2018;6(6):432-436.
8. Minh BQ, Nguyen MAT, Von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Molecular biology and evolution. 2013;30(5):1188-1195.
9. Montezano DG, Sosa-Gómez DR, Specht A, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SD, *et al*. Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. African entomology. 2018;26(2):286-300.
10. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and evolution 2015;32(1):268-274.
11. Pashley DP. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Annals of the Entomological Society of America. 1986;79(6):898-904.
12. Rathna Priya TS, Nelson ARLE, Ravichandran K, Antony U. Nutritional and functional properties of coloured rice varieties of South India; c2019.
13. Reineke A, Karlovsky P, Zebitz CPW. Preparation and purification of DNA from insects for AFLP analysis. Insect molecular biology. 1998;7(1):95-99.
14. Sharanabasappa S, Kalleshwaraswamy CM, Poorani J, Maruthi MS, Pavithra HB, Diraviam J. Natural enemies of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a recent invasive pest on maize in South India. The Florida Entomologist. 2019;102(3):619-623.
15. Shobha A, Manjulakumari D, Geetha Bali. An inexpensive technique for mass rearing of pod borer, *Helicoverpa armigera* (Hubner). Eds. Shakuntala Sridhara, Nagachaitanya B, Chakravarthy AK and Prabhakara Shetty TK. Recent Trends in Animal Behaviour. New India Publishing agency, New Delhi; c2009. p. 209-219.
16. Shylesha AN, Jalali SK, Gupta ANKITA, Varshney RICHA, Venkatesan T, Shetty PRADEEKSHA, *et al*. Studies on new invasive pest Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae) and its natural enemies. Journal of Biological Control. 2018;32(3):1-7.
17. Srinivasan R, Othim STO. Fall armyworm in Africa: which 'race' is in the race and why does it matter?; c2018.
18. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Molecular biology and evolution. 2021;38(7):3022-3027.
19. Trifinopoulos J, Nguyen LT, Von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic acids research. 2016;44(W1):W232-W235.