Original Research Article

Molecular characterization of fall armyworm (*Spodoptera frugiperda*, J.E. Smith) feeding on rice (*Oryza sativa* L.) in Tamil Nadu

ABSTRACT

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an invasive insect pest of India native to the Western Hemisphere. The pest damages more than 350 plant species. FAW is genetically divided into two strains *viz*. corn (C) strain which feeds mostly on maize, sorghum, *etc*. and the other is a rice (R) strain that prefers rice and other small grasses. In the present study, fall armyworm samples were collected from rice crop in two different places of Tamil Nadu, India during 2019-20 and strain identity was confirmed. Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) profile of mitochondrial *cytochrome oxidase* subunit I fragment exhibited the presence of both 'R' and 'C' strains of FAW in rice crop. Nucleotide variations were observed from PBS, TNAU isolate (ON247930) at five places and two places in Killikulam isolate (OM491244).

Keywords: Fall armyworm; Rice; Molecular characterization; Strain; PCR-RFLP; Phylogenetic analysis

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most significant grains and staple foods. More than 60 per cent of the world's population is reliant on it to meet their nutritional needs [1]. It is grown on over one-fourth of the total agricultural area in India, feeding more than half of the country's population [2]. In Tamil Nadu, it is the predominant food grain crop grown in an area of 20,36,239 hectares with a production of 68,81,725 tonnes. Thanjavur district is the largest rice growing district, covering 2.14 lakh hectares, followed by Tiruvarur (1.95 lakh hectares), Tiruvannamalai (1.71 lakh hectares), and Ramanathapuram (1.34 lakh hectares) [3].

More than hundred insect species attack rice plants, with twenty of them capable of causing economic damage. The most common insect pests include stem borers, gall midge, plant hoppers, leaf fodders, rice hispa, gundhi bug, and case worm [4]. Recently, the maize fall armyworm (FAW), *Spodoptera frugiperda*, J.E. Smith was found infesting rice crop in two different places of Tamil Nadu. In fact, FAW is a recently introduced pest in India and very quickly it has invaded variety of crops like sorghum, pearl millet, finger millet, barnyard millet, sugarcane, turmeric, fodder grasses, ginger, fodder crops *etc*. [5,6,7].

FAW races can only be differentiated molecularly, but not morphologically [8,9]. The corn-designated 'C' strain, feeds mostly on maize, sorghum, cotton, and pulses, and the rice-designated 'R' strain, prefers rice and other grass species, are two genetically distinct races of FAW [10,11]. Mitochondrial *COI* markers are commonly used to identify and assess population structure in insects, and they are known for

maternal inheritance, high copy number, and conservative nature, among other things [12]. The use of restriction enzymes in PCR-RFLP analysis of the mitochondrial *COI* gene aids in the identification of fall armyworm strains [13,14]. In this context, the present study was done to identify FAW strain(s) that feed on rice through molecular approaches.

2. MATERIALS AND METHODS

2.1. Insect collection and preservation

FAW larvae (5 per location) were collected from rice crop in two distinct locations, as shown in table 1. Individually collected insects were kept in microfuge tubes and stored at -20 ^{o}C until DNA extraction.

2.2. DNA preparation and quantification

The CTAB method (cetyl trimethylammonium bromide) was used to isolate the genomic DNA of FAW [15]. DNA was isolated from larvae's prolegs. Tissues were homogenised completely in 200 µl of extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4M NaCl, 0.02M EDTA, 2% CTAB, and 0.2 per cent mercaptoethanol), then kept at 65°C for an hour before centrifugation at 12,000 rpm for 15 minutes. An equal volume of chloroform:isoamyl alcohol (24:1) was added to the supernatant and centrifuged at 10,000 rpm for 10 minutes to separate the phases. The top aqueous phase was transferred to a 1.5 µl sterile microfuge tube and the process was repeated. To precipitate the DNA, 200 µl of ice-cold isopropanol was added to the aqueous phase, which was then kept at -20 ^{o}C overnight. After centrifugation at 10,000 rpm for 10 minutes at 4 ^{o}C, the DNA pellet was recovered. The DNA pellet was air dried after being

washed twice with 100 % ethanol. The pellets were suspended in 30-50 µl of 1X TE buffer and kept at -20 ^{o}C until PCR analysis. Nanodrop Spectrophotometer (Genova, USA) and agarose gel electrophoresis were used to examine the extracted DNA quantitatively and qualitatively.

2.3. PCR analysis

DNA extracted from FAW larvae was PCR amplified. DNA samples were diluted in 1X TE buffer to obtain a 50-100 ng/µl working solution (working aliquot). Bioserve Biotechnologies Private Limited, Hyderabad, India, produced the primers. *MtCOI* region was amplified using the primer pairs JM 76F (5' GAGCTGAATTAGG(G/A)ACTCCAGG 3') and JM 77R (5' ATCACCTCC(A/T)CCTGCAGGATC 3'), producing a 568-bp fragment. All segments were PCR amplified with a 25 µl reaction mixture (12.5 µl of 2X Thermo Scientific PCR Master Mix, 1 µl forward primer, 1 µl reverse primer, 9.5 µl nuclease free water, and 1 µl DNA template) in an Eppendorf thermocycler with an ambient thermo-cycling profile of 94 ^{o}C (1 min), followed by 33 cycles of 92 ^{o}C (45 s), 56 ^{o}C (45 s) 72 ^{o}C (1 min) and a final extension of 72 ^{o}C for 3 min. On a two per cent PCR grade agarose horizontal gel, PCR amplified products were fractionated and documented using the Bio-Rad Gel Doc XR imaging equipment.

2.4. RFLP analysis

To determine strain identity, PCR products of the *COI* region were digested with *Sac*I restriction enzyme purchased from Thermo Fisher Scientific and New England Biolabs, respectively. A reaction mixture including 10 µl PCR product, 2 µl buffer, 1 µl

enzyme and 17 µl nuclease free water was prepared, incubated at 37 °C for 2 hours, fractionated on a 2% agarose gel, and documented.

2.5. Sequencing and GenBank submissions

The unpurified PCR amplified product (20 µl) was shipped to Bioserve Biotechnologies India Pvt Ltd. in Hyderabad, Telangana, India, for double-pass DNA sequencing. The raw sequence was aligned and edited using Bioedit software (version 7.25). A dendrogram was produced by aligning the partial nucleotide sequences of *MtCOI* from *S. frugiperda* using Clustal W, and a phylogenetic tree was constructed using MEGA11 (version 11.0). A bootstrap analysis with 1000 replications utilizing the neighbour joining method was done to determine the evolutionary distance between the sequences [16].

3. RESULTS

3.1. Strain identity analysis using PCR-RFLP

The PCR analysis results revealed the expected fragment size of about ~568 bp from *mtCOI* region was amplified in all the tested samples (Fig. 1A). Restriction enzymatic analysis of *mtCOI* segments exposed that, *Sac*I enzyme cut the DNA into ~413 bp and ~155 bp fragments in Rice (R) strain whereas, DNA remain uncut (~568 bp) in Corn (C) strain (Fig. 1B). Out of ten, eight samples showed ‘R’ strain identity and two samples collected from Paddy Breeding Station, TNAU, Coimbatore, India assumed ‘C’ strain identity. But interestingly, our FAW samples (two numbers) showed ‘C’ strain identity.

3.2. Nucleotide variations

Nucleotide variations were found at different places in the *mtCOI* region of FAW populations feeding on rice (Fig. 2). Highest variation in the nucleotides was observed from PBS, TNAU isolate (ON247930) at five places *viz*. 108 ('A' is replaced by 'T'), 159 ('T' is replaced by 'C'), 390 ('C' is replaced by 'T'), 464 ('C' is replaced by 'T'), 470 ('T' is replaced by 'C') and 501 ('T' is replaced by 'C'). The Killikulam isolate (OM491244) showed nucleotide variations in two places *viz*. 112 ('A' is replaced by 'T') and 515 ('A' is replaced by 'T').

3.3. Phylogenetic tree analysis

The data matrix included 29 sequences from *MtCOI* genome of two taxa: one taxon includes 28 FAW sequences, of them 13 across the globe and 15 from India including our test sequences (Fig. 3). The taxon, *S. exigua* served as an out group. The phylogenetic tree built using maximum livehood (ML) following Tamura 3-parameter model showed that, the tree was divided into two main branches in that, first main branch was further divided into two clades. First clade contained majority of FAW sequences was clustered and closely related to *S. frugiperda*. Our collection (one sample) from Killikulam, India (OM491244) was grouped in this branch. The second clade clearly showed it was diverged from other sequences due to substitution of nucleotides in our PBS, TNAU, India (ON247930) isolate of FAW.

4. DISCUSSION

Genetic polymorphisms are currently the most extensively used and most reliable way for identifying races within species. Mitochondrial haplotypes are generally used

with those defined by polymorphisms in the *cytochrome oxidase* subunit I gene (*COI*) the best characterized [17].

According to mtCOI analysis, FAW feeding on rice from two different locations in Tamil Nadu were found to have both the 'R' and 'C' strains. Eight of the ten samples were found to be 'R' strain, with two being 'C' strain. Studies by Nagoshi and Meager [17] implied that, the rice strain populations of FAW prefer to feed on rice, grass species *etc*. and corn strain populations prefer corn and sorghum for feeding. Opposing results to our findings were also known to occur. Irrespective of host plants, FAW reported on maize, sweet corn and sorghum assumed ‘R’ strain identity with minimal genetic diversity exhibiting no host/ location specific variations [18].

In our study, nucleotide variations were observed in both the test sequences. Studies by Swamy *et al*. [18] revealed nucleotide variations at four (254, 305, 540, 588) and 14 positions (72, 117, 171, 207, 253, 258, 305, 489, 540, 564, 570, 589, 600, 634) when compared with the NCBI accessions U72977 and U72974 for ‘C’ strain of FAW collected from maize, sweet corn and sorghum crops in India.

In a dendrogram, the FAW population feeding on sugarcane from Anakapalle formed a distinct clade with 99.75 percent similarity to the Mexico population with the 'C' strain identification [19]. Chormule *et al*. [6] reported that the FAW is found in sugarcane crops in Kolapur, Maharashtra, and that it is of the 'C' strain with close resemblance to USA FAW populations (U72974, U72975 and U72976). Our isolate from Killikulam, India (OM491244) was grouped in major sequences with ‘R’ strain identity whereas,

TNAU, India isolate (ON247930) totally diverged from these sequences and grouped in the ‘C’ strain sequences.

5. CONCLUSION

In India, the 'R' strain has colonized maize, sorghum, sweet corn, rice and a variety of other crops, while the 'C' strain is also adapting to sugarcane, finger millet, pearl millet and now in rice. As a result, both strains have migrated to India, where they have colonized a wide range of crops. Hybrid strains (R/C or C/R) could form from the interbreeding populations of both strains, feeding on a wide range of hosts and causing damage to a variety of agricultural crops.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS DISCLAIMER:

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Table 1. Details on the FAW collected from rice in different locations of Tamil Nadu

S. No.	Location	Life stage	Sample size	Date of collection	GPS information	NCBI Accession number(s) received
1.	Paddy Breeding Station, TNAU, Coimbatore, India	Larval	05	20.10.2020	Latitude: 11.01˚N Longitude: 76.94˚E	ON247930
2.	Agricultural College and Research Institute, Killikulam, Vallanad, Tuticorin, India	Larval	05	24.09.2020	Latitude: 08.71˚N Longitude: 77.86˚E	OM491244

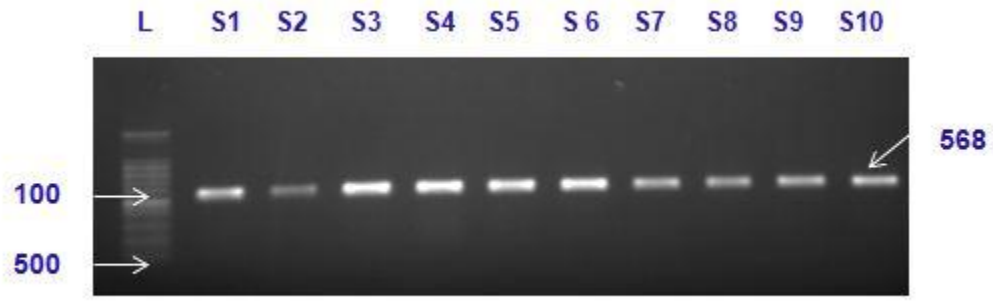

Fig. 1A. Agarose gel displaying PCR products of FAW collected from rice

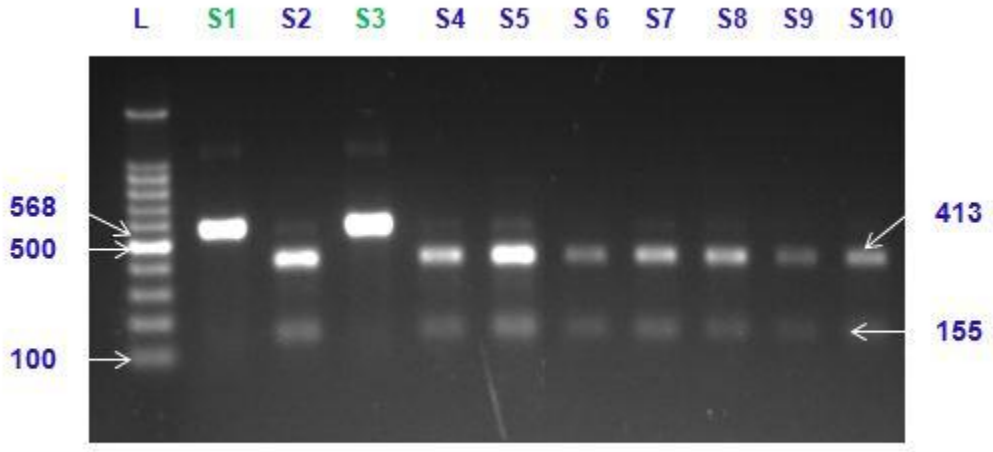

Fig. 1B. Strain specific RFLPs from the mitochondrial *COI* gene *Sac*I digestion

Lane L– Ladder (100 bp); Lanes S1-S5 – PBS, TNAU, Coimbatore, India; Lanes S6-S10 – AC&RI, Killikulam, Thoothukudi, India

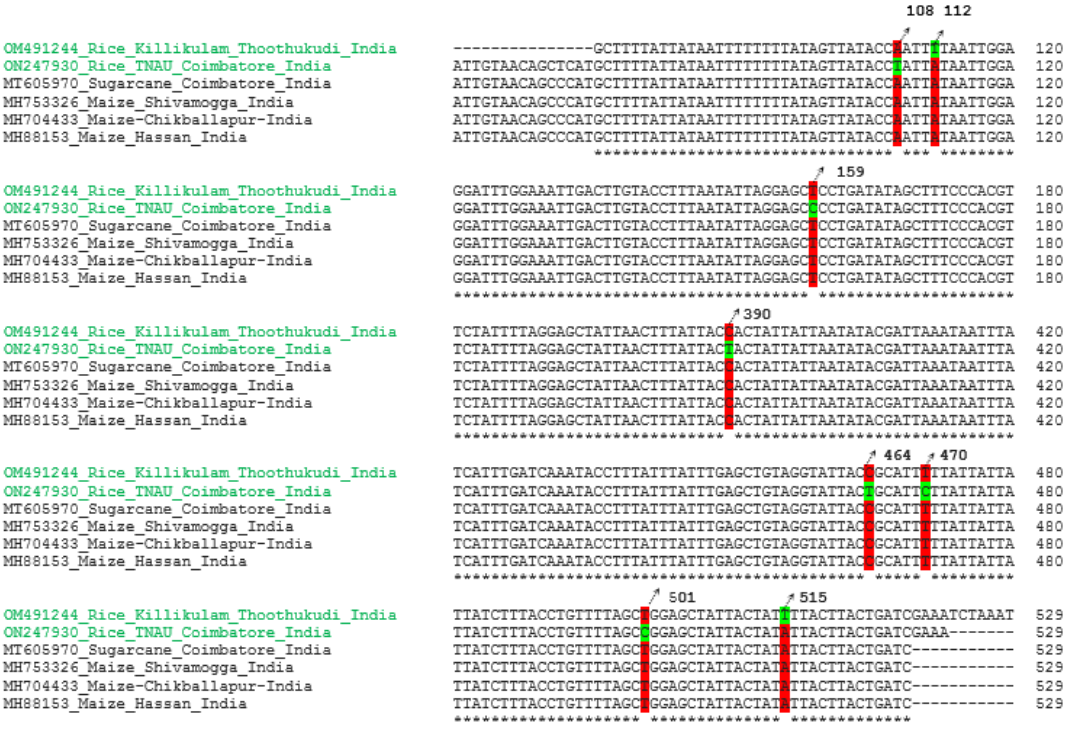

Fig.2. Nucleotide variations in the test and other FAW sequences

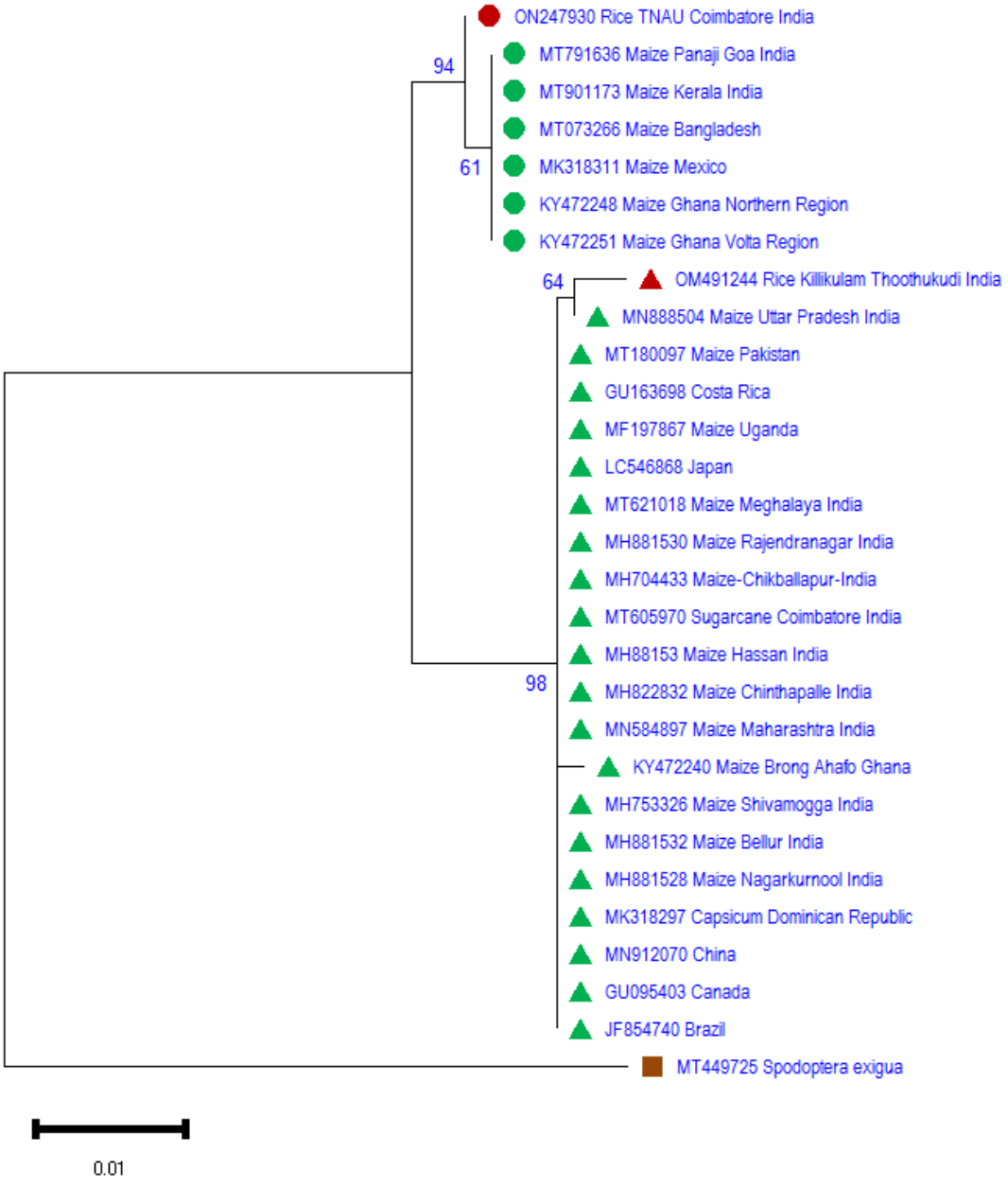

Fig. 3. Phylogenetic tree showing the relationships among the fall armyworm strains. Bootstrap support is indicated on the branches.