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First report of the Fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India

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ABSTRACT: The global agriculture often faces new threats from invasive alien insect pests, pathogens, weeds etc requiring immediate attention and co-operative action to manage the pestilence. In this regard, the fall armyworm (FAW), *Spodoptera frugiperda* is a notorious pestiferous insect with high dispersal ability, wide host range and high fecundity that make it one of the most severe economic pests. The FAW has been restricted to the Americas and recently in 2016 reported from various countries in Africa, posing a serious challenge of sustainability in Sub-Saharan African countries. Herewith we report the occurrence of the FAW on maize in various districts of Karnataka state, India. Identification of FAW has been carried out employing morphological and DNA barcoding. Phylogenetic analysis has revealed that FAW clustered with Florida (rice strain), Ghana, Nigeria, Uganda on maize. India predominantly being a tropical country favours high rate of multiplication round the year and its high pestiferous nature poses a formidable challenge to Indian agriculture warranting immediate action before it assumes a serious proportion.

Keywords: Fall armyworm, *Spodoptera frugiperda,* India, maize

INTRODUCTION

Invasive alien species (IAS) pose a serious threat to agriculture and cost billions of dollars in terms of reduced production and productivity. A recent study showed that about 1300 species of invasive insect pests and pathogens have been introduced into 124 countries (Paini *et al*., 2016). This is mainly due to increased transboundary movement of agricultural commodities, anthropogenic activities, climate change etc. (Paini *et al*., 2016). Our recent surveys and further identification lead to confirmation of the entry of one more new invasive pest, Fall Armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae) into India. It is native to the tropical and subtropical region of America, where it is a serious pest of corn but also known to attack more than 100 hosts. In addition, it is reported to cause major damage to economically important cultivated grasses such as rice, sorghum, and sugarcane as well as horticultural crops like cabbage, beet, tomato, potato and onion besides cotton, pasture grasses, peanut, soybean, alfalfa and millets (Pogue, 2002; Chapman *et al*., 2000; CABI, 2016). Till 2015, FAW has not been reported other than the Americas. But a severe incidence of FAW was reported from African countries such as São Tomé, Nigeria, Bénin and Togo in 2016 (Goergen *et al*., 2016) and later in Ghana during 2017 (Cock *et al*., 2017). Subsequently, it has spread to most of the sub-Saharan Africa, where it is causing extensive damage, especially to maize and to a less extent on sorghum and other crops. Within a short span of its introduction in Africa, FAW has been confirmed in over 43 African countries (Prasanna *et al*., 2018). It is reported to cause a 34% reduction in grain yield (Lima *et al*., 2010) and annual loss up to US dollars 400 million in Brazil (Figueiredo *et al*., 2005).

First observations of fall armyworm, *S. frugiperda* were made in early May-June 2018 in maize fields

at College of Agriculture, Shivamogga, Karnataka, India. Further, its presence was recorded in different districts of Karnataka. During the first fortnight of June 2018, following repeated calls by maize growers and the Department of Agriculture, Davanagere District, a survey was conducted to assess the incidence and confirm its presence in diffrent districts. However, the damage was initially attributed to cutworms, *Spodoptera litura* Guenée, and *Mythimna separata* (Walker) which occurred in outbreak form during 2017 *kharif* season. Herewith we report the occurrence of *S. frugiperda* in different parts of Karnataka confirmed through morphological and DNA barcoding.

MATERIALS AND METHODS

Sample collection

Survey for the occurrence of *S. frugiperda* was conducted in different maize growing areas of Karnataka. Larvae were collected from the following locations.

SHIVAMOGGA (Southern transition zone): College of Agriculture, 13° 58.44' N, 075° 34. 52' E, coll. Sharanabasappa; Muttodu, 13° 58.36' N, 075° 35' E, 580 MSL, coll. Kavita Hegde.

DAVANAGERE (Central dry zone): Lellakatte, 14° 29' N, 76° 03'E, 662 MSL, coll. Sharanabasappa ; Hosagodu, 14° 34' N; and 76° 09'E; 611 MSL, coll. CM Kalleshwaraswamy; Devikere, 14° 30.448' N, 076° 14.113' E, 661 MSL, coll. ST Prabhu.

CHITRADURGA (Central dry zone): Hullehal, 14° 18' N, 75° 54'E, 731 MSL, coll. Pavithra H B; Basapur, 13° 58' N, 75° 34'E, 687 MSL, coll. Sharanabasappa.

CHIKMAGALURU (Central dry zone): Chicknayakanahalli (Kadur taluk), 13° 57' N, 76° 10'E, 715 MSL, coll. CM Kalleshwaraswamy (on sweet corn).

CHAMARAJANAGAR (Southern dry zone): Chandakavadi 11° 54' N, 77° 00'E, 694 MSL, coll. Shivaray Navi; Hanur 12° 08' N, 77° 30'E, 694 MSL, coll. Shivaray Navi.

BELAGAVI (Northern dry zone): Tukanatti 16° 18' N, 74° 51'E, 596 MSL, coll. MS Maruthi**.** A large number of larvae collected were brought to the laboratory, reared to adults and observed for diagnostic characters of *S. frugiperda* through morphological and molecular approach. Infestation level was recorded in each of the localities surveyed.

Species identification

Male Genitalia examination

Male genitalia was dissected (Clark, 1941) and compared with earlier findings (Pogue 2002 and EPPO, 2015). Dissection of male genitalia was performed with Zeiss-Stemi 508 stereo binocuar microscope and imaged using a Leica M-205C Auto Montage stereomicroscope. Voucher specimen used and dissected genitalia in labelled micro vials with glycerin was housed at the Department of Entomology, College of Agriculture, UAHS, Shivamogga.

DNA extraction

Total genomic DNA was extracted from *S. frugiperda* larvae using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Black and Duteau, 1997) with some modification and was used for PCR analysis. PCR analysis did for an expected size of approximately 700 bp of COI 5 prime region using universal barcode primers LCO1490 (5'- GGTC AAC AAA TCA TAA AGA TAT TGG -3') and HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA -3') (Folmer et al 1994). The 25 µl PCR reactions for *mtCOI* consisted of 3 uL of the Genomic DNA sample, 1 ul of 10 mM dNTP, 0.7 ul of 10pM primer, 2.5 ul of 10× reaction buffer, 0.7 ul of 25mM Mgcl2, 0.3 ul of DMSO, 1 ul of 2 unit of Taq DNA polymerase (Thermo scientific). PCR reactions were performed in a thermal cycler (Eppendorf, CA). PCR reactions were pre-incubated for 5 min at 95 °C followed by 30 cycles of 50 s at 94 °C; 40 s at 48 °C; 45 s at 72 °C. Samples were finally incubated for 10 min at 72 °C followed by chilling at 8 °C. The PCR negative control contained the identical amount of PCR mixture with 3 µl of distilled water instead of DNA template. A PCR positive control was also included, containing the PCR mixture plus DNA that had been successfully put through the PCR reaction on previous studies. To ensure repeatability, three replications for each of the reactions were conducted. These three replicates were kept separately for further sequencing reactions. PCR amplification products were separated by electrophoresis in a 1.5% agarose gel in TAE buffer (40 mM Tris-acetate (pH 8.00), 1 mM EDTA) at 100 V for 1 hour. The gel was then visualized under ultraviolet light. The PCR amplified fragments were eluted using NucleoSpin® Extract II Kit (MN) according to the manufacturer's protocol. The standard recombinant DNA techniques used in cloning (Sambrook, 2001). The *E. coli* DH5α cells were transformed with PCR-amplified ~700 bp mtCOI gene ligated in pTZ57R (T/A cloning vector) using DNA ligation kit (#K1214). The transformed cells

(20µl) were spread on LB agar plates containing X-gal (270 µg/ml), IPTG (120 µg/ml) and ampicillin (100 µg/ml). The plates were then incubated at 37˚C for 24 h to screen blue and white colonies. Cloning was confirmed by colony PCR, plasmid mobility check and restriction analysis of recombinant plasmid DNA containing the *S. frugiperda* mtCOI gene. Isolated plasmid DNA, from an overnight bacterial culture, using a reliable plasmid miniprep method was sequenced using standard M13 sequencing primers. *Spodoptera frugiperda* species MtCOI gene fragments were sequenced using purified recombinant plasmids. Sequencing was performed in triplicates of the above clones and the PCR products in an automated sequencer (ABI Prism® 3730 XL DNA Analyzer; Medauxin, Bengaluru).

Chromatograms were edited to discard ambiguous bases and edited sequences were aligned by using the Basic Local Alignment Search Tool (BLASTn, http://www.ncbi.nlm.nih.gov), with the sequences of same or related genera retrieved from the nucleotide database (PUBMED) of National Centre for Biotechnology Information (NCBI). Sequences containing insertions, deletions, nonsense or stop codons were considered as having resulted from PCR/sequencing errors or represented pseudogenes and were thus excluded from the analyses. *In silico* analysis of the sequences were carried out to confirm the sequence accuracy. COI sequences corresponding to different geographical location and hosts of *S. frugiperda* species used in this study were downloaded from the National Centre for Biotechnology Information (NCBI) GenBank for phylogenetic analysis. Sequence alignment was performed employing the CLUSTAL W multiple alignment tools in BioEdit. 7.0.5.3. sequence alignment editor. The sequences were further analyzed using MEGA.6.0 (Tamura et al., 2013) to obtain conspecific and congeneric distances, whilst Neighbor-Joining trees were constructed using the Kimura-2-parameter (K2P) distance model (Kimura, 1980; Saitou and Nei, 1987) employing MEGA.6.0(Tamura *et al*., 2013). All the sequences generated in the present study were deposited in NCBI-GenBank.

Phylogenetic trees were reconstructed by using Tamura-Nei model (Tamura *et al*., 2013) for COI sequences and Kimura 2-parameter model (Kimura, 1980) and graphically displayed in a maximum likelihood (ML) tree by the program MEGA 6.0 (Tamura *et al*., 2013). To assess the phylogenetic support for groupings on the tree, we performed a bootstrap resampling analysis (2,000 replications).

RESULTS AND DISCUSSION

Morphological characters of male and females of *S. frugiperda* were studied. The male has brown forewing with contrasting markings, a small conspicuous white spot at junction of M3 and CuAl veins, a white patch at apex (Plate 2). Whereas, female had a small faint marking on forewing (Plate 3). Male genitalia with uncus curved in apical half, slender and pointed at apex; Valve quadrate, ampulla slightly curved, clavus short; costal process narrow, inclined in the middle, elongate (Plate 4). The coremata with single lobe on each side (figure not shown). The male genitalia dissected was similar to earlier reports (Pogue, 2002 and EPPO, 2015) strongly suggesting that the species is *S. frugiperda*.

Sequence analysis

Fragments amplified from the MtCOI primer pairs aligned to their target reference sequence. All the search analysis results revealed that the analyzed species belongs to *S. frugiperda*. Alignment of the *S.frugiperda mtCOI* sequences found to have no deletions or insertions and no stop codons, consistent with the amplified DNA arising from functional COI genes.

The *S. frugiperda MtCOI* DNA sequences from Indian population were identical to each other, and they were 87-100% identical compared to Sequences from other countries. BLASTn search of DNA barcodes from Indian specimens revealed 98-99% nucleotide sequence identity with *S. frugiperda* voucher specimens with USA, Ghana, Nigeria, Uganda and Canada maize population. Whereas the Barcode of life Data base identification results shows that Indian maize fall armyworm belongs *S. frugiperda* sp. 1 with 99.66% similarity.

S.frugiperda sequences from India were deposited with NCBI Gen Bank.

Phylogenetic analysis

Phylogenetic tree generated from the *S. frugiperda mtCOI* data from this study alone, did not show any major clades or differences. The present phylogenetic analysis consisted of 36 *S.frugiperda* MtCOI sequences including 5 generated from this study, 36 sequences were retrieved from the NCBI Genbank database which include the sequences across the world and one sequence of *Bombyx mori COI gene* with NCBI accession number EU141360 served as an outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei parameter model (Tamura *et al*., 2013). The tree with the highest log likelihood (-2202.3048) is shown. The percentage of trees in which the associated taxa clustered together is

Plate 1. Larva

Plate 2. Male

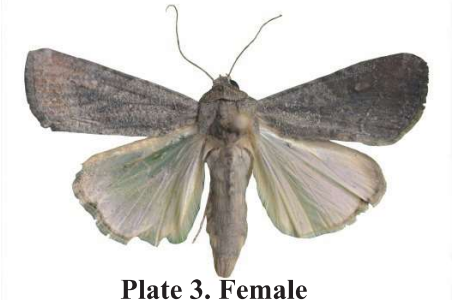

Plate 3. Female

Plate 4. Male genitalia

shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree generated by combining the NCBI Genbank sequences showed 2 major clades (Fig 3). All the *S. frugiperda* Indian Maize population were clustered in clade1 which had a high degree of geographical representation consisting of Uganda, Ghana, Nigeria Maize population and USA Maize and rice populations, indicating a possible infestation of other crops like rice, sorghum and vegetables. Clade 2 represented the populations from Nigeria, Uganda, Netherlands and USA maize population. This might be due to the high conservation of the amino acid sequences in the *S. frugiperda* mitochondrial genomes. It has been reported that *S. frugiperda* occurs in two races, a 'Rice strain' (R strain) and a 'Corn strain' (C strain) (Pashley *et al*., 1985). The rice strain preferentially feeds on rice and various pasture grasses and the latter feed on maize, cotton and sorghum, although this may be geographically variable (Hebert *et al*., 2003). It is not clear at this juncture, whether both the strains are introduced to India, haplotype analysis is required for further confirmation. Our results of both morphological and molecular data confirmed the occurrence of *S. frugiperda* in India. This constitutes the first report of its presence from India.

Our field observation demonstrated its establishment in parts of Karnataka state. The crop stand was 15-30 days old in most of the localities surveyed. On an average, 10-20 per cent infestation was noticed in all the districts surveyed. Feeding by the early instars resulted in white elongated patches and later instars caused windows on leaves and faecal pellets in the whorls (Plate - 1). As *S. frugiperda* known to prefer maize than any other hosts, the impact of loss on maize in India, particularly in Karnataka state is going to be enormous in the years to come. The reason being, maize is the third most important food crops after rice and wheat in India. The area under maize is 9.26 mha with a production of 23.67 m tones (Anonymous, 2016). Amongst the Indian states, Karnataka stands first in area and production. The conditions prevailing in India may suit *S. frugiperda* and chances of its establishment are stronger. In temperate states of North America, *S. frugiperda* arrives seasonally and then dies out in cold winter months. But in much of Africa, continuous generations throughout the year have been reported due to tropical and subtropical climate (Prasanna *et al*., 2018). As much of India falls under tropical climate, the occurrence of *S. frugiperda* throughout the year is expected.

In the past three years, at least four species of insect pests have invaded India affecting agricultural production.

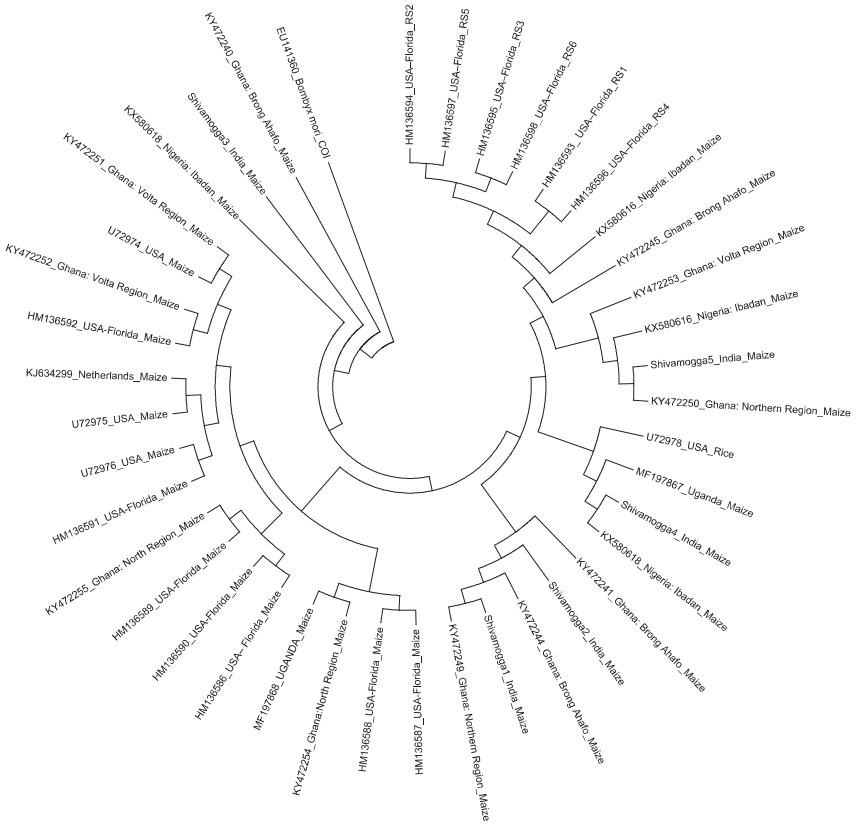

Figure 3. The phylogenetic relationships of 5 samples, from India Maize population, inferred from the 658 bp mitochondrial cytochrome oxidase I (COI) sequence using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993)**.** Bootstrap values (2,000 replications) reproduced in <60% replicates were collapsed and the bootstrap values are shown at the branch points. The GenBank accession numbers of the corresponding reference s equence are indicated. The analysis involved 36 nucleotide sequences and there were a total of 723 positions in the final dataset. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st+2nd +3rd+Noncoding. All positions containing gaps and missing data were eliminated. The nucleotide sequences were aligned using the MUSCLE (27) and the evolutionary analyses were conducted in MEGA 6.0 (Tamura and Nei, 1993).

Some of the recent invasive insect pests reported in India were Western Flower Thrips*, Frankliniella accidentalis* (Pergande) (Kamud and Vikas, 2015), South American Tomato leafminer, *Tuta absoluta* (Meyrick) (Sridhar *et al*., 2014, Kalleshwaraswamy *et al*., 2015, Shashank *et al*., 2015), Coconut rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Sundaraj and Selvaraj, 2017) and Neotropical solanum whitefly, *Aleurothrixus trachoides* (Back) (Sundararaj *et al*., 2018). Addition of *S. frugiperda* to this list has the enormous impact on the Indian agriculture.

At this point, the port of entry of the fall armyworm into India is not yet determined but could be deciphered based on trade analysis and employing haplotype analysis. One of the possibilities could be through the import of agricultural commodities. As this pest is reported to have remarkable dispersal capacity, high reproductive capacity, the absence of diapause and wide host range, it is likely to spread to the entire country and further to other neighbouring countries. Hence, there is urgency for nationwide coordinated efforts to contain the pest.

Other than maize, the pest is currently found feeding on sorghum, sweet corn and baby corn in natural conditions (personal observation). In captivity, the larvae were found feeding on cabbage, tomato, groundnut

and sugarcane but not on rice. This is another concern as the pest may shift to other hosts and survive in the absence of maize and maintain the population in India throughout the year. Immediate focus of research could be on reproductive biology, host preference and host plant resistance. The present investigation may help the researchers to throw a light on the above said issues.

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