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FALL ARMY WORM *SPODOPTERA FRUGIPERDA* STRAINS IN GOA AND ITS INCIDENCE ON FODDER MAIZE

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ABSTRACT

Fall army worm (FAW) *Spodoptera frugiperda* (J E Smith) is a highly destructive invasive insect pest. Its incidence was observed during post kharif season 2019 at Old Goa, and it ranged from 43 to 83% @ 0.67 larvae/ plant with a maximum of 1.16 larvae/ plant during vegetative stage, and it was more than the reproductive stage. The mtCO1 analysis of the populations from Goa revealed the presence of both Rice (R) strain and Corn (C) strain which feed on fodder maize and sweet corn, and this confirms the prevalence of both the strains in Goa on fodder maize.

Key words: *Spodoptera frugiperda*, Old Goa, fodder maize, invasive pest, mtCO1, R and C strains, incidence, host plants, vegetative and reproductive stages, post kharif

Fall army worm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is a highly voracious insect pest native to tropical and subtropical region of Americas. It has a wide host range of >353 host plants (Montezano et al., 2018). The most frequently damaged plants are maize, sorghum, rice, millet, soybean, peanut, cotton, Sudan grass and other fodder grasses. 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 also reported in neighbouring countries like Nepal and China (Ratna et al., 2019; FAO 2019). The incidence on maize ranged from 9.0 to 62.5% and 6 to 100% (Shylesha et al., 2018; Mallapur et al., 2018) in different districts of Karnataka. This has 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 populations in India have revealed that majority of these were 'R' strain- feeds on maize, sorghum and sweet corn; while 'C' strain- feeds on sugarcane (Mahadevaswamy et al., 2018; Chormule et al., 2019). The knowledge on its strains and genetic diversity is important for developing IPM strategies (Srinivasan et al., 2018). Hence, the present study to explore the presence of FAW and its strains in the state of Goa on different host plants and to assess the damage incidence on fodder maize.

MATERIALS AND METHODS

Fodder maize variety African Tall was sown with the spacing of 50x 20 cm during post kharif season 2019 at the experimental farm of ICAR- Central Coastal Agricultural Research Institute, Ela, Old Goa, Goa. The area of experimental field was 1000 m^2 from which 30 plants were randomly selected and weekly observations were made on the number of larvae/ plant and plants damaged, assessed based on the damage symptoms viz., skeletonizing the upper epidermis, windows on leaves and faecal pellets in the whorls. To know the status of FAW strains in Goa, larvae were collected from the experimental field at ICAR as well as in other locations on different host plants viz., fodder maize, sweet corn and water melon. Larvae were placed in 1.5 ml micro centrifuge tubes separately. A single larva was selected and ground in a pestle and mortar using liquid nitrogen. About 25 mg of the ground powder was used to isolate the genomic DNA by using Wizard Genomic DNA purification kit (Promega Corporation, USA Cat. A1120) as per the manufacturer's instruction. The remaining individuals were preserved as voucher specimens at -70°C in ICAR-CCARI, Goa. PCR Amplification of a 658 bp region near the 5' terminus of the CO1 gene from the genomic DNA using primers (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and (HCO 2198 5'-TAAACTTCAGGGTGACC AAAAAATCA-3'). PCR reaction was carried out with a 20µl reaction mixture containing 1.0 µM of each primer, 10 µl master mix (Promega Corporations)

and 50ng of the DNA template in Mastercycler Pro (Eppendorf, GmBH) thermal cycler with the following conditions. Initial denaturation of 94°C for 5 min; 35 cycles of 94°C for 60 sec, 50°C for 60 sec, 72°C for 60 sec and final extension of 10 min at 72°C. The amplicon was visualised on 1.2% agarose gel containing 0.5 µg/ml of ethidium bromide the amplified product was purified using Qiagen Mini elute PCR purification kit (Qiagen India) and quantified using Nano drop-1000 (Thermo fisher scientific, USA). The purified fragments were sequenced by M/S Eurofins Genomics India Pvt. Ltd., Bengaluru, India. Sequences were edited manually and aligned using Clustal W and submitted in NCBI GenBank. Phylogenetic analysis was performed using MEGA-X (Kumar et al., 2018) by using neighbor-joining (NJ) and the algorithm of maximum composite likelihood with 1000 bootstrap re-samplings. The phylogenetic tree was generated using mtCO1 sequences of 45 *S. frugiperda* from the NCBI Genbank database which include the sequences from India, from across the world and representing both 'R' and 'C' strains.

RESULTS AND DISCUSSION

The data reveals that FAW larval counts and damage incidence varied in different growth stages, ranging from 0.67 to 1.16 larvae/ plant during vegetative stage, with more larvae in the vegetative stage compared to the reproductive stage. Damage in vegetative to reproductive stage revealed that 18.72 plants got infested/ 1000 m^2 amounting to 43 to 83% with maximum being during vegetative stage. In India, severe damage had been reported in various states 9.0 to 62.5% in Karnataka, 56 to 92% in Rajasthan and 16 to 52% on fodder maize in Goa (Shylesha et al., 2018; Meena et al., 2019; Maruthadurai and Ramesh, 2020). Jaramillo et al. (2019) in different phenological stage of corn found more larvae in the vegetative stage than in the reproductive stage.

There are two morphologically identical host strains of FAW that are defined by their host plant preferences but can be distinguished by molecular techniques (Pashley et al., 1985). The BLAST analysis of the sequences took into account all the NCBI GenBank sequences and obtained accession numbers of the mtCO1 DNA sequences (MT791628 to MT791636). The analysis revealed that the Goa populations were 99% identical to the sequences from India and other countries. Phylogenetic tree of mtCO1 gene sequences was generated using the neighbor joining (NJ) algorithm and the phylogenetic tree carrying boot strap values is presented in Fig. 1. Further sequence analysis for mtCO1 5' revealed the presence of both 'R' strain and 'C' strains in Goa. Seven isolates (S1, S2, S3, S4, S5, S6 and S7) were aligned with 'R' strain and two isolates (S8 and S9) were aligned with 'C' strain. The previous studies on isolate MK368810 was found aligning with 'R' strain (Maruthadurai and Ramesh, 2020). Nucleotide variations between 'R' strain and 'C' strain are observed in 11 positions (34, 79, 133,169, 220, 451, 526, 532, 562, 596, 625) when 'R' and 'C' strains of Indian and global population was analysed (Table 1). Similar results were reported by Mahadeva Swamy et al. (2018). Periodical sampling during the entire crop period, revealed the presence of 'R' strain and 'C' strain in Goa on fodder maize which indicated that both strains of *S. frugiperda* have been occurring in the Coastal state of Goa. These results derive support from those of Bhavani et al. (2019) in which the analysis indicated the presence of 'R' strain and 'C' strain in sugarcane at Anakapalle in Andhra Pradesh; also with those of Chormule et al. (2019) on the presence of 'C' strain

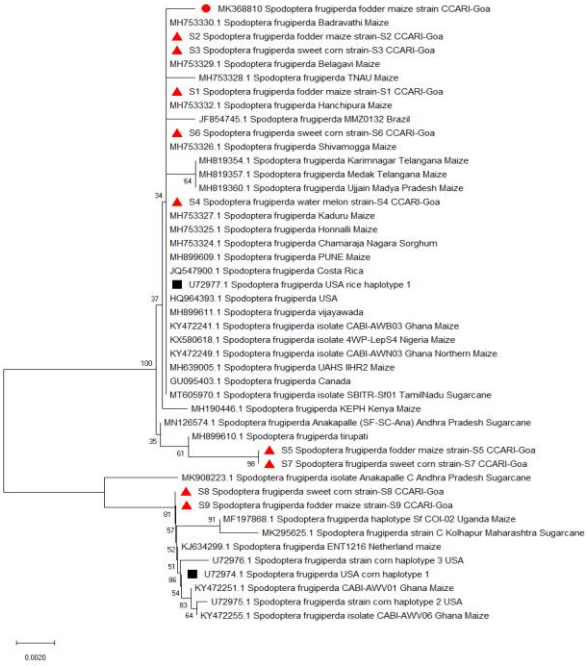

Fig. 1. Phylogenetic neighbor-joining tree based on the partial 5'mtCO1 gene sequences of isolates of *S. frugiperda*; isolates from this study represented by red triangular block, previously reported 'Rice' strain from Goa is represented by red circular block and 'Rice' and 'Corn' reference strains by a square block. All others strains are reference strains obtained from the GenBank database for phylogenetic analysis. The tree was generated by MEGA-X software using the NJ and the algorithm of Maximum composite likelihood with 1000 bootstrap re-samplings. Numbers at each branch indicate bootstrap value. Scale bar represents 2 nucleotide substitutions/ 1000 nucleotides.

Table 1. Position-wise nucleotide variations in 5' terminus of mtCOI gene of FAW of "Rice strain" and "Corn strain"

Nucleotide position in the 5' mtCO1	Rice strain	Corn strain
34	A	G
79	A	G
133	C	T
169	A	T
220	T	C
451	C	T
526	C	T
532	T	C
562	T	C
596	C	T
625	A	T

Total number of strains used is 45 (34 rice, 11 corn strains)

on sugarcane in Maharashtra. Nagoshi et al. (2007) reported that both 'R' and 'C' strains feed on maize and other crops during the same crop period in Brazil. Nagoshi and Meagher (2004) compared the distribution of two strains from corn fields before and after harvest and found that the 'C' strain constitutes 72 and 39%, respectively. However, molecular diversity studies of Indian populations of FAW collected on maize, sweet corn and sorghum from six states of the country revealed the prevalence of 'R' strain (Mahadeva Swamy et al. 2018). It appears that 'R' strain has colonized maize, sweet corn and sorghum while 'C' strain has started adapting to sugarcane, fodder maize and sweet corn. Thus, the present study reports the presence of 'R' strain and 'C' strain of FAW from Goa on fodder maize and sweet corn. It also provides basic information on damage potential and larval density on fodder maize.

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