Molecular characterization of the Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize in southern Rajasthan, India

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Abstract

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a polyphagous Lepidopteran pest, a native to tropical and sub-tropical America and recently it has invaded the African and Asian countries. Presently, the mitochondrial *Cytochrome Oxidase Subunit* I (*COI*) - gene based molecular characterization of FAW samples from the maize fields of southern Rajasthan has revealed the occurrence of corn and rice strains there. The occurrence of such *S. frugiperda* population of Rajasthan region could be traced its origin from the Florida-Caribbean region or African region. Further, the *Tpi* gene region analysis showed that the *S.frugiperda* forms found in the maize fields are only the corn strains. In the Indian Rajasthan populations of FAW, the *Tpi*-variant2 category is the highest one and is then followed by the *Tpi*-variant1 and *Tpi*-variant3 was unique with C and T at *Tpi*e4$_{192}$ and *Tpi*e4$_{198}$, respectively. Further research is needed towards the confirmation of these tentatively identified strains of *S. frugiperda* that would in turn helpful for the proper monitoring, host-plant identification and the effective management of such pests.

Introduction

An invasive pest, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a tropical American insect that is responsible for huge economic loss (Prasanna et al. 2018). This pest had invaded Central and Western Africa during early 2016 and due to its voracious feeding nature and rapid migratory behavior it could able to rapidly spread across the Sub Saharan countries that have caused severe damages there (Goergen et al. 2016). As it is a serious pest, it could cause severe damage to almost 100 plant species representing the 27 families including the maize- crop and other graminaceous crops like millet, sorghum, rice, wheat and sugarcane (Goergen et al. 2016) and thus it would pose threat to the food security of the millions of people (De Groote et al. 2020). Montezano et al. (2018) have also reported a host range of 353 plant species representing 76 plant families like; Poaceae, Asteraceae and Fabaceae from Brazil. Over 30 countries have reported its occurrence within their borders including the island countries (Prasanna et al. 2018). The yield losses in maize ranging from 8.3 M to 20.6 M tons per year have been reported from 12 maize producing countries (CABI 2016). Fall armyworm attack occurs in maize crop right from emergence to tasseling, silking and cob formation stage. This pest can reduce corn grain yield up to 34% and is estimated at U$400 million loss annually in Brazil (Sena et al. 2003; Lima et al. 2010).

In 2018, FAW has been reported in many Asian countries like India, Yemen, Thailand, Myanmar and Sri Lanka (Liu et al. 2019), Bangladesh (Chhetri and Acharya 2019) and subsequently its spread has been reported in China, Korea, Japan and Australia (Zhang et al. 2019; Guo et al. 2018; Lee et al. 2020; Acharya et al., 2021). In India, the FAW was first time reported in Karnataka state, on maize, in May, 2018 (Sharanabasappa et al. 2018) and subsequently, within a year, the pest was found to spread in the other states *viz.*, Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Tamil Nadu, Telangana, West Bengal (Ramesh Babu et al. 2019), Arunachal Pradesh, Meghalaya, Manipur, Sikkim, Mizoram, Nagaland and Tripura (Firake et al. 2019). In Rajasthan, we have reported this pest for the first time in early 2019 from the Southern region of the state on winter maize

which was confirmed by the morphological and molecular characterization (Ramesh Babu et al. 2019). However, its infestation was already been reported in some of the popular hybrids, inbreds of maize and sweet corn in the region.

The FAW species consists of morphologically identical but genetically distinct subpopulations namely the corn strain (CS) and the rice strain (RS) (Pashley et al. 1985; Prowell et al. 2004). While the corn strain's preferred feeds are; maize, sorghum and other grasses, the rice strain prefers rice and other large grasses (Pashley et al. 1986). Both these strains are region- specific and they differ in their response to bioagents like Bt and various groups of insecticides and they also exhibit different dispersal patterns (Pashley et al. 1992). Pest management strategies are formulated based on such differences between the two strains (Adamczyk et al. 1997).

Molecular genetic markers are one of the most reliable methods to differentiate the diverse strains of FAW although their morphometric features can be analyzed based on their wing shapes (Cañas-Hoyos et al. 2014). The allozymes, esterases, PCR-RFLP (DNA amplification and digestion), FR (For Rice) fragment repeats and AFLPs have been generally used to differentiate the corn strain (CS) with that of rice strain (RS) in FAW (Pashley 1986; Levy et al. 2002; Nagoshi and Meagher 2003; Prowell et al. 2004; Groot et al. 2010). Mitochondrial cytochrome oxidase subunit I (*COI*)- gene sequence was shown polymorphic variation between C and R strains whereas, the populations collected from the maize fields having an R-strain in the mtDNA COI gene region (Acharya et al. 2021). Mahadeva Swamy et al. (2018) have recently studied the molecular aspect of FAW and reported the prevalence of RS FAW in maize (using mtDNA gene regions). In the present study, we have used molecular markers based on the partial regions of *Cytochrome Oxidase Subunit I* (*COI*) and also another genetic marker, the nuclear *triosephosphate isomerase (Tpi)* gene which is linked with Z-chromosome to confirm the species identification, differentiate the corn and rice strains and the origin of haplotypes. The single nucleotide polymorphisms (SNPs) in the *Tpi* gene region perhaps could form one of the reliable methods to detect the strain identity than the mtDNA *COI* gene regions (Nagoshi et al. 2017) and which could be also helpful to find out the host plant preference by the two strains.

Materials And Methods

During the present experiments, the standardized procedures of Levy et al. 2002; Ve'lez-Arango et al. 2012; Laura Juarez et al. 2012; Nagoshi et al. 2017 and 2018 were followed to detect FAW strains in Rajasthan populations and compared the COI haplotype pattern with the other region (Western and Eastern) populations.

DNA Extraction

Fall armyworm larvae were collected from the maize fields at various places of Rajasthan (India) during *Rabi* and *Kharif* seasons, 2019 (January to September 2019) (Table 1). The collected specimens were

preserved at -20^{o}C. Total genomic DNA was extracted using DNA Sure Tissue mini kit (Nucleo-pore, Genetix Brand, India) by following the manufacturer's instructions.

Table 1
Collection information of FAW, *Spodoptera frugiperda*

Code Name	Location of region	Collection year	Source
BSW	Borwat Farm, Banswara Rajasthan, India	2019	Maize fields
PTG	Cheniyakheri, Pratapgarh Rajasthan, India	2019	Maize fields
DPRP	Punawara, Dungarpur, Rajasthan, India	2019	Maize fields
DPRM	Malmata, Dungarpur, Rajasthan, India	2019	Maize fields
RJD	Kunwara, Rajsamand, Rajasthan, India	2019	Maize Fields
SLB	Salumber, Rajasthan, India	2019	Maize fields
UDR	RCA Farm, Udaipur, Rajasthan, India	2019	Maize fields
CTG	Bassi, Chittorgarh, Rajasthan, India	2019	Maize fields

PCR Amplification of COI regions:

Presently, all the PCR reactions were carried out by using C1000Touch™ Thermal cycler of Bio-Rad, USA. The PCR amplification was performed for 50µL containing 25 µL DreamTaq PCR Master Mix (2X) (Thermo Fisher Scientific Inc.), 2µL of template DNA, 10 pmol of each forward and reverse primers (Table 2) and final volume was made by using nuclease free water. PCR was performed according to the different primers used with initial denaturation for 4 min at 94°C, followed by 35 cycles of 30 sec denaturation at 94°C, 45 sec primers annealing at 47°C, 45 sec initial extension at 72°C and a final extension of 20 min at 72°C (Ramasubramanian et al. 2016). The amplified PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide (0.5µg/µL) for 60 min at 80 V (BIO-RAD, USA) and visualized in gel documentation system (Gel Doc™ EZ Imager, BIO-RAD, USA).

Table 2
Primer used for the identification of fall armyworm species, *S. frugiperda* in maize

Primer name	Forward primer & Reverse primer	Reference
Universal primers COI-I	LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Folmer *et al* (2013)

PCR purification, sequencing and analysis

The PCR products were purified by using GeneJET PCR purification Kit (Thermo Fisher, Scientific Inc.) and sequenced by using ABI PRISM 3730xl Genetic Analyzer by Applied Biosystems, USA (Agile Life science Technologies India Pvt. Ltd, Pune). The obtained sequences were aligned through BioEdit sequence alignment editor (version 7.0.5.3) and homology was confirmed by using National Center for

Biotechnology Information (NCBI)- Basic Local Alignment Search Tool (BLAST) (BLASTn, http://www.ncbln1m.nih.gov). A phylogenic tree was graphically constructed in MEGA x program (Kumar et al. 2016).

FAW Genotyping (FAW strain analysis): Restriction Site Length Polymorphism (RFLP)

The PCR reaction was carried out for the amplification of *Cytochrome Oxidase Subunit* I (*COI*) gene of ~ 600 bp, using the forward primer JM76 (5'-GAGCTGAATTAGG(G/A)ACTCCAGG-3') and the reverse primer JM77 (5'-ATCACCTCC(A/T)CCTGCAGGATC-3') to find out the two mitochondrial haplotypes of *S. frugiperda* based on the RFLP, according to the methods of Levy et al. (2002); Nagoshi and Meagher. (2003a); Laura Juarez et al. (2012); Cano-Calle et al. (2015).

Characterization of the other CO1 regions and Tpi gene region

Presently, we have also used another *COI* primer (apart from the universal *COI* primers) to confirm and determine the strains (Corn and Rice strain) of *S. frugiperda*. To determine the regions specific haplotypes and the host strain identity, another *COI* region was amplified with the primers 891 F or 893 F and 1472 R or 1303 R (Table 3). To identify the host strain and region-specific haplotypes, the COI-II primers 891F and 1472R were used (Nagoshi et al. 2012 and 2017). The SNPs analysis at COI-II 1164 (T) and COI-II 1287 (A) identifies as rice-strain whereas, four corn-strain haplotypes (h1-h4) were found for the corn-strain group. Polymorphisms in the genetic marker, the *Triosephosphate isomerase* gene (*Tpi*) also used to identify and confirm the two strains of FAW and comparable with the *CO1* gene markers. *Tpi* region was amplified by using primers 412 F and 850 R (Table 3). Based on the single nucleotide polymorphism (SNPs) at *Tpi* e4183 the two strains of *S. frugiperda* can be identified, where, the C-strain (CS) contains C nucleotide at 183 (*Tpi* e4183-C) and the R-strain (RS) contains T nucleotide at 183 (*Tpi* e4183 T) (Jing et al. 2019).

Table 3
Primers used for the identification of host-specific strains and haplotypes of fall armyworm, *S. frugiperda* in maize

Primer name	Forward primer & Reverse primer	Reference
COI –II	*891 F*: 5'-TACACGAGCATATTTTACATC-3'	Nagoshi et al (2017) & 2018
	1472 R: 5'-GCTGGTGGTAAATTTTGATATC-3'	
COI –III	*893 F*: 5'-CACGAGCATATTTTACATCWGCA-3'	Nagoshi et al (2012)
	1303 R: 5'-CAGGATAGTCAGAATATCGACG-3'	
Tpi	*412 F*: 5'-CCGGACTGAAGGTTATCGCTTG-3'	Nagoshi et al (2017) & 2018
	850 R: 5'-AATTTTATTACCTGCTGTGG-3'	

Data in the GenBank

The obtained gene sequences were submitted/deposited to/in the GenBank and the following accessions were obtained: Universal primers COI-I (MK633906; MK591010; MN486491-95; MN117927); COI –II (MZ377090- MZ377097); JM 76 & 77 primers (MT185359-185360; MT189266-271); COI –III primers (MZ388549-MZ388550); Tpi primer (MZ418118- MZ418125).

Results

The *COI* gene sequences of *S. frugiperda* from Banswara (BSW), Dungarpur (DPRP), Chittorgarh (CTG), Salumber (SLB), Udaipur (UDR) and Pratapgarh (PTG) regions were 98–100% identical with the sequences from India (Vijayawada: GenBank MH899611 and Tirupati: GenBank MH899610 of India) and from other countries (Dominica Republic: GenBank MK3182971; Kenya: GenBank MH190445; South Africa: GenBank MF593258). A BLASTn search of DNA barcodes for another Dungarpur (DPRM) (GenBank No. MN486491) and Rajsamand (RJD) (GenBank No.MN486493) FAW populations revealed 100% nucleotide sequence resemblance with Kenyan sample (GenBank No. MH190448) and Bangladesh (GenBank No. MT073266). The sequences were 99–100% identical in sequence data and coverage. Phylogenetic analysis revealed that out of eight samples collected from maize in different places of the region, six were clustered to the *S. frugiperda* rice-strain haplotype RS9 whereas the two were clustered to the corn-strain haplotype CS1 of the United states and also with African strains, Indian strains and also with China region corn-strains (Fig. 1).

The PCR-RFLP analysis revealed that the two fragments of about 510 and 90 bp size were obtained due to cut by *Msp*I of PCR fragments for the corn strains but not by *Sac*I whereas PCR fragments of the rice strain cut by *Sac*I showed a reciprocal pattern by producing 450 and 150 bp fragments, but not by *Msp*I (Fig. 2). The corn and rice strain nucleotides differences are given in Fig. 5. In another analysis, the *Eco*RV enzyme digested PCR products derived from the primers COI-II: *COI*-893F/*COI*-1303R showed a single band for the corn strains (DPRM and RJD) whereas, strains of other regions showed typical diagnostic two bands which is a characteristic of rice strain (Fig. 3). The single band PCR products were purified and sequenced to confirm the absence of *Eco*RV site. Based on the COI-III: *COI* primers 891F/1472R, the FAW samples from RJD and DPRM regions were having similar polymorphic sites (G_{1164}; G_{1287} haplotype) which are designated as corn strains. Similarly, the six regions of Rajasthan displayed the polymorphic sites at T_{1164}; A_{1287} and identified as Rice strains (Table 4). The analysis of the *Tpi* strain markers revealed that all the FAW populations collected from maize fields at Rajasthan were C-strain (*Tpi*-C haplotype) as defined by *Tpi* $e4_{183}$ (Table 5). In Indian Rajasthan populations, *Tpi*-variant2 category is the highest then followed by *Tpi*-variant1 and Tpi-variant3 is unique with C and T at *Tpi*$e4_{192}$ and *Tpi*$e4_{198}$, respectively. The comparison between INDRJ *Tpi*-variants with *Tpi*-R category identified four different sites (Tpie4_{129}, Tpie$4_{144,}$ Tpie4_{168} and Tpie4_{180}) in addition to the strain diagnostic site (*Tpi*183).

Table 4

Comparison of single nucleotide difference between India (IND) FAW populations with consensus sequences of USA (US) and Africa (AF) rice and corn haplotypes

Haplotype/Strain	COI-II							Corn strain haplotypes:
	1125	1164	1176	1182	1197	1216	1287	US-CS
INDRJ-CS	T	G	T	C	G	T	G	$A_{1164;}$ A_{1287} haplotype 1 (h1); A_{1164}; G_{1287} haplotype 2 (h2)
INDRJ-RS	C	T	C	T	A	A	A	(Predominate in South America and Texas)
US-CS	T	R	T	C	G	T	R	
US RS	C	T	C	T	A	A	A	G_{1164}; A_{1287} haplotype 3 (h3);
AF-CS	T	G	T	C	G	T	G	G_{1164}; G_{1287} haplotype 4 (h4)
AF-RS	C	T	C	T	A	A	A	(Florida and Greater Antilles)

INDRJ-India Rajasthan present in bold and italic form; CS-Corn strain; RS-Rice strain; US-America; AF-Africa; INDRJ-RS-present in BSW;UDR; DPRP; PTG; SLB;CTG

Table 5
Tpi corn and rice strain FAW haplotypes comparison of Indian with the Western Hemisphere (W.H.) and African populations

Tpi haplotypes	**Tpi single nucleiotide polymorphisms (SNPs)***							
	129	144	165	168	180	183	192	198
Tpi-CS*						C		
Tpi-RS*						T		
Tpi-CS catergory (W.H. variants and Africa variants)*								
Tpi- C1	C	G	C	T	C	C	C	C
Tpi- C2	C	G	C	T	C	C	T	T
Tpi-RS catergory (Africa Variant)*								
Tpi-R	T	A	C	C	G	T	C	C
Tpi-RS catergory* (W.H. Variants)								
	C	G	T	C	C	T		
	C	G	T	C	T	T		
	C	G	C	T	C	T		
	C	G	A	C	C	T		
	C	G	G	C	C	T		
	A	G	T	C	C	T		
	C	A	T	C	C	T		
	C	G	C	C	T	T		
	C	G	G	C	T	T		
INDRJ variants								
Tpi variant 1	C	G	C	T	C	C	T	T
Tpi variant 2	C	G	C	T	C	C	C	C
Tpi variant 3	C	G	C	T	C	C	C	T

Adopted from Nagoshi et al (2018) CS-Corn strain; RS-Rice strain; INDRJ-India Rajasthan; Indian variants are bold and italic

Discussion

In the present study, the molecular markers of both Tpi and COI genes were used to characterize the populations of FAW collected from the maize fields of Rajasthan region. The *COI* gene sequence analysis by using universal primers revealed that all the specimens collected from the maize were of *S. frugiperda* and a similar *COI* gene fragment was earlier reported by Shylesha et al. 2018 and Mahadeva Swamy et al. 2018. The specimens collected from different regions of Rajasthan revealed that the two types of haplotypes are present in the FAW populations. This has been represented in the phylogenetic tree analysis where out of eight populations, six were clustered with the fall armyworm rice-strain specific haplotypes and two with corn-strain specific haplotypes. Earlier, Nagoshi et al. (2017) have also observed similar types of haplotypes *i.e.*, corn and rice strains from Togo, Africa based on the *COI* region analysis. Their sequences were identical with US corn and rice-strain haplotypes. As per the present study, both the rice and corn strains are prevalent in the Indian region and they are identical to the Western hemisphere as well as with the Eastern hemisphere. In another earlier study by Nagoshi et al. (2019) found one Ecuador haplotype clustered with the rice-strain and others were similar to the corn-strain haplotype (when they analyzed the *COI* gene sequences). Jing et al. (2019) have studied the phylogenetic tree which was constructed by using the Maximum likelihood method and which revealed that the majority of FAW populations belong to the corn-strain haplotype in China. Similarly, in Korea, Lee et al. (2020) reported the incidence of *S.frugiperda* in maize and they analyzed the haplotypes by using *COI* gene which comprised of two haplotypes based on the phylogenetic analysis.

Molecular markers have been used to differentiate the corn and rice strains of *S. frugiperda*. In the present study, PCR-RFLP method was adopted by using JM76 and JM77 primers and the amplified COI region PCR products were digested with the restriction enzymes, *MspI* and *SacI*. Out of the eight populations from different regions of Southern Rajasthan, six (BSW;UDR;CTG;PTG;DPRP;SLB) and two populations (DPRM;RJD) were identified as rice strains and corn strains, respectively. Earlier also, Machado et al. (2008) have used several molecular markers to differentiate the strains of *S. frugiperda* and they used the PCR-RFLP of the mitochondrial gene *Cytochrome Oxidase Subunit I* (*COI*) and the PCR of the tandem repeated fragment FR. Based on a PCR-RFLP product of 569 bp of the COI gene that is digested with restriction enzymes, *Msp*I and *Sac*I. Two cleavage sites of 497 and 72 bp were observed in the corn strain when PCR products were digested with *Msp*I (Levy et al. 2002; Nagoshi and Meagher 2003), whereas when the enzyme was *Sac*I, two cleavage sites of 500 and 69 bp have been observed in the rice strain (Lu et al. 1994).

The analysis of another *COI* region by using the primers *COI*-893F/*COI*-1303R generated a PCR product having a single *Eco*RV site which is a characteristic of the rice strain. PCR products of the two regions Dungarpur (DPRM) and Rajsamand (RJD) didn't have the *Eco*RV site which was confirmed by the sequencing of the PCR products and confirmed that the FAW populations belong to the corn strain. Nagoshi (2019) revealed that the *Eco*RV recognition site in the COI-RS haplotype and this strain-specific polymorphism site were not present in COI-CS haplotype in African populations.

Nagoshi et al. (2007 & 2015) reported the geographically divided sub-groups for the corn strains of *S. frugiperda* based on differences in the mitochondrial haplotypes derived from polymorphisms at sites

COI$_{1164}$ and *COI*$_{1287}$ in the Western hemisphere. The western hemisphere contains most of the TX-type profile whereas FL-type belongs to Florida and Caribbean regions. Populations of corn strains collected from Togo (African country) revealed the differences in haplotype pattern which was most consistent with the pattern of FL-type. In the present study, similar *COI* region primers 891F/1472R were used to study the haplotypes of *S. frugiperda* in maize. The analyzed COI-CS FAW populations were from Rajsamand (RJD) and Dungarpur (DPRM) regions that had $G_{1164}G_{1287}$ SNP configuration which is most consistent with the Florida-Caribbean region and Africa and Togo region corn haplotyes (Nagoshi et al. 2017). The FAW populations present in the Southern Rajasthan may be the source or originated from Sub-Saharan African region or Florida-Caribbean region.

Single Nucleotide Polymorphisms (SNPs) in the genetic marker and sex-linked *Triosephosphate isomerase* (*Tpi*) gene could also help in identifying the host-specific strains which could provide more accurate identification information of the host specific strains of *S. frugiperda*. The results of this gene can be comparable with the COI gene markers for confirmation (Nagoshi et al. 2017 & 2018). Earlier, based on the results of such studies Nagoshi reported the presence of two strains of *S. frugiperda* in the Western as well as Eastern Hemisphere. *Tpi* coding region was PCR amplified using the *Tpi* primers 412 F and 850 R. The e4183 SNP varies as C or T for C-strain allele (*Tpi*-C) is indicated by a C183 or the R-strain (*Tpi*-R) is by T183. Based on this the African FAW populations showed 60% and 65% corn strains based on *COI* and *Tpi* markers. This indicates that the corn strains have been predominant in the FAW populations of African countries which were similar to that of Western hemisphere populations based on the *Tpi* gene markers. Based on the *Tpi* marker, all FAW populations collected from maize at Southern Rajasthan revealed that all of them were of corn-strain based on the polymorphism in the *Tpi* e4$_{183}$ site. In Indian Rajasthan populations, apart from two *Tpi* variants one unique variant3 was found in the FAW populations.

The present analysis of mtDNA *COI* gene regions confirmed that both the rice and corn strains are present in different regions of maize fields of Southern Rajasthan. The same set of FAW populations were tested with *Tpi* marker revealed that the corn-strain haplotypes are predominant in the collected populations of *S. frugiperda* from maize fields of Rajasthan. This indicates *Tpi* marker-based corn-strain populations showed expected associations with the host plants ie. maize than the mitochondrial markers. Therefore, this study gives a preliminary idea of the occurrence/presence of two strains of *S. frugiperda* and it is rare/unusual that both the strains have been present in the samples of maize plants of Rajasthan. A more detailed survey has to be undertaken over some time by covering different regions of Rajasthan and also from different host plants. So that the exact origin and strains of *S. frugiperda* will be identified and that would have a positive impact on the timely management strategies of this pest in the future.

Declarations

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Author contributions

Dr. S. Ramesh Babu contributed to the study conception and design. Material preparation, data collection and data analysis were performed by Sonika Joshi, M. Raja, Deepika Kalyan, P. Swathi, S. Ramesh Babu, R. K. Kalyan,

P. Rokadia and Beerendra Singh. The first draft of the manuscript was written by Dr. S. Ramesh Babu and all authors commented on previous versions of the manuscript. Dr. P. Perumal along with all authors read, reviewed, revised, approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Figures

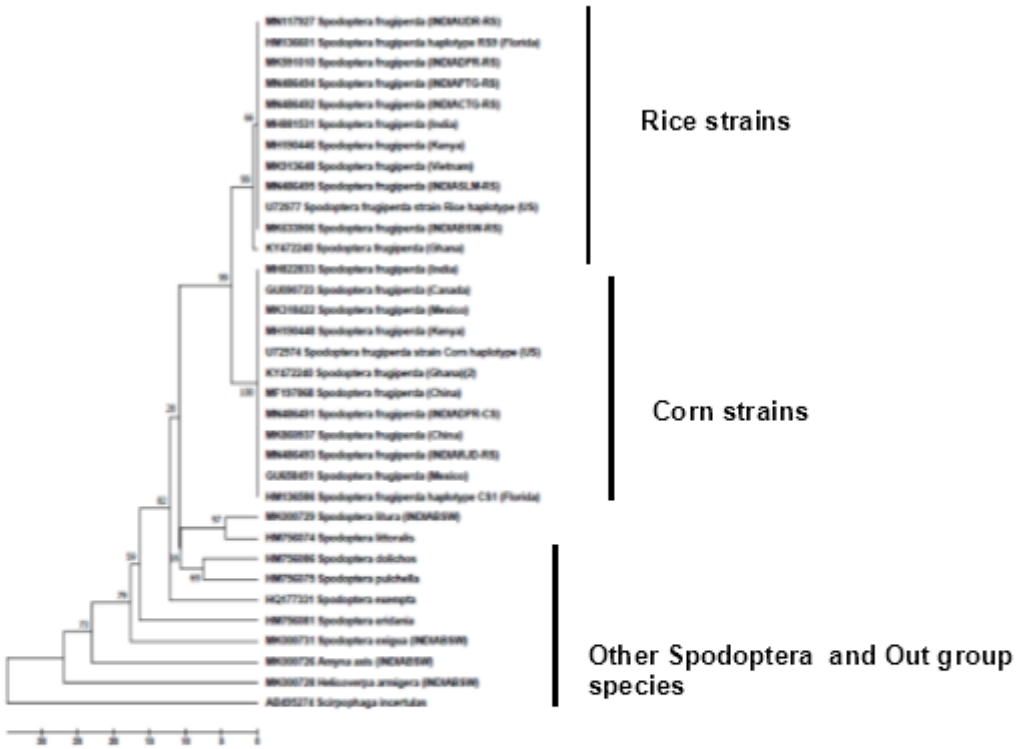


Fig. 1 The analysis involved 34 nucleotide sequences and conducted in MEGA 6. The evolutionary history was inferred using the UPGMA method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. (Spodoptera species found in GenBank: *S. dolichos* (HM756086); *S. littoralis* (HM756074); *S. exempta* (HQ177331); *S. eridania* (HM756081)

Figure 1

[See figure]

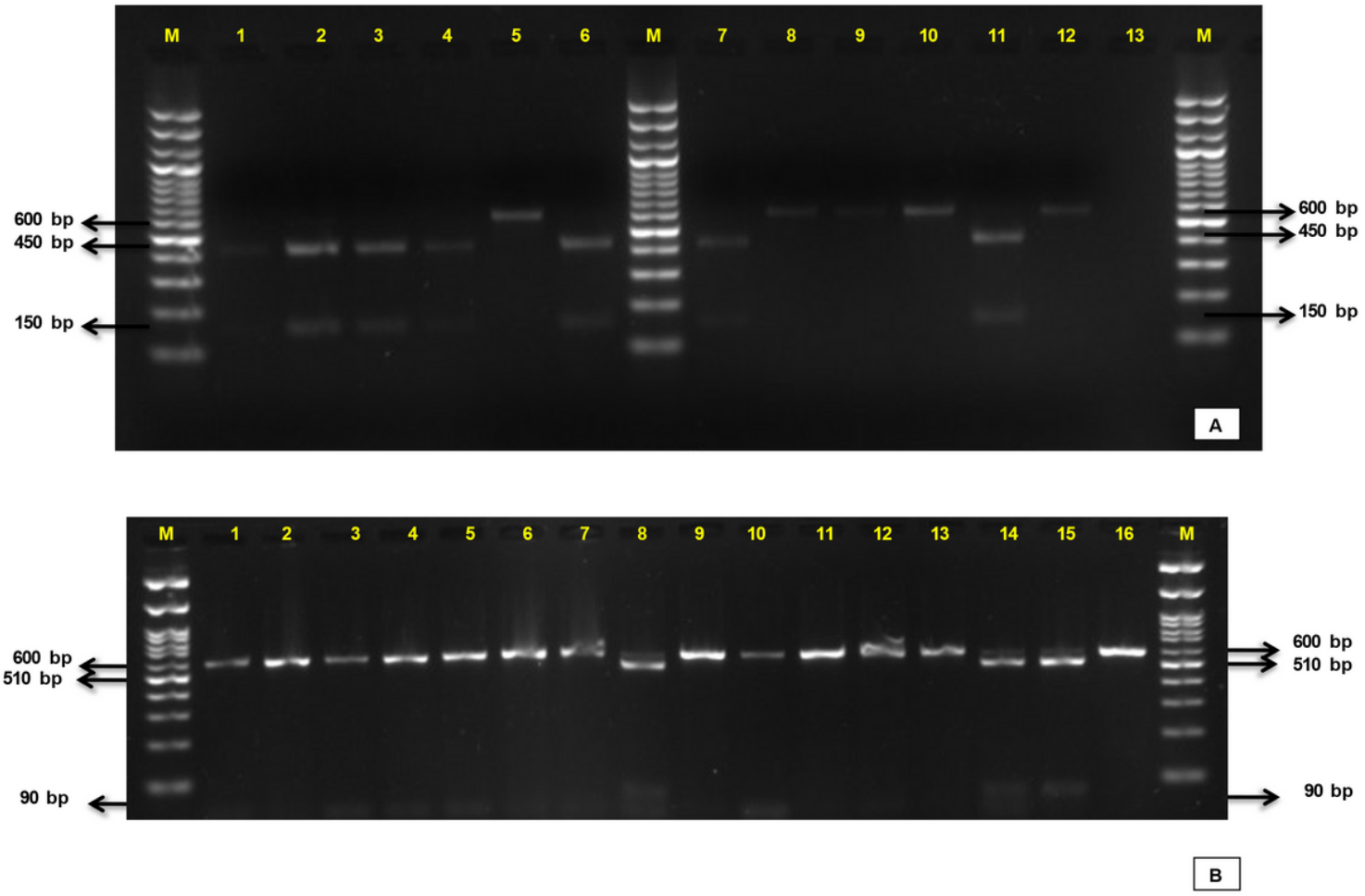
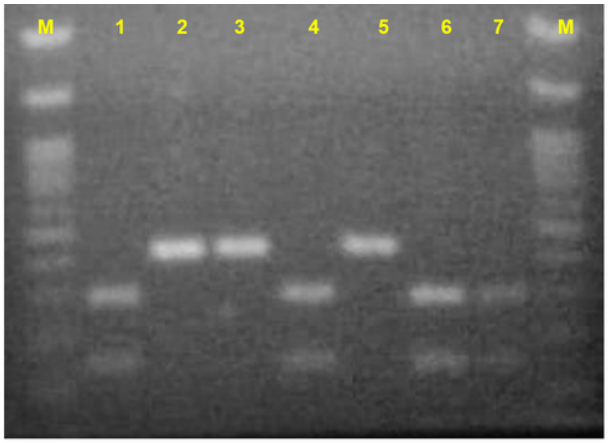


Fig. 2 PCR amplification of *COI* gene of *S. frugiperda* and digested with restriction enzymes, *Msp*I and *Sac* I
*A. Sac*I enzyme digested samples (Rice strain) ; (1 -4, 6, 7,11,=rice strains) 13 -Control) (without DNA)
B *Msp*I enzyme digested samples (Corn strain); (8,14 and 15 Corn strains) 600 bp= uncut fragment; M=molecular marker. (100 bp);

Figure 2

[See figure]



3a Rice strain (Chittorgarh (CTG))

AAAATATGCTGTACCAACAGGGTATTAAAATTTTTAGTTGATTAGCTACTTTCCATGGAACTCAAATCAATTATTCCCCATCTATTTTATGAAGATTAGGA
TTTGTATTTTTATTTACTGTAGGGGGATTAACAGGTGTAATTTTATCTAATTCTTCTATTGATATTACTTTACATGATACTTACTATGTAGTTGCTCATTTC
CACTATGTTTTATCAATAGGAGCTGTATTTGCTATTTTAGGTGGATTTATTCACT **GATATC** CATTATTTACTGGATTATCTTTAAATCCTTATATATTAAAA
ATTCAATTTTTTATTATATTTATCGGAGTAAATTTAACTTTCTTCCCACAACATTTTTTAGGATTAGCAGGTATACCTCGTCGATATTCTGAACTATCCTGA
A

3b Corn strain (Dungarpur (DPR1))

ACAAATTTGCTGTCCACGGGTATTAAAATTTTTAGTTGATTAGCTACTTTCCATGGAACTCAAATTAATTATTCCCCATCTATTTTATGAAGATTAGGATTT
GTATTTTTATTTACTGTAGGAGGATTAACAGGTGTAATTTTATCTAATTCTTCTATTGATATTACTTTACATGATACTTACTATGTAGTTGCTCATTTCCAT
TATGTTTTATCAATAGGAGCTGTATTTGCTATTTTAGGGGGATTTATTCATTGATACCCATTATTTACTGGGTTATCTTTAAATCCTTATTTATTAAAAATT
CTATTTTTTATTATATTTATCGGAGTAAATTTAACTTTCTTCCAACAGCATTTTTTAGGGTTATCAGGTATACCTGCGCGATATATGCGACTGTTCAGATA
CATTAAAGTGTTTGTAGCATACATAGGCCGGCAGAATGATCACGTTAAAGAGTCCACNNCCNACGTACATTAAGTGCACAGGGGTAAAATGGATGATC
AGAGCGTGCACATGCCTCAGAAGGTCAACTACTACTCGTTTCAAAACTCGATAATGATCCATCCTCAGGTTACTTTA

Fig. 3 Gel picture shows PCR amplification of *ECOR*V digested *COI* gene (region of *S. frugiperda* [Double band Rice strains: 1, 4, 6, 7); Single band Corn strains; 3 and 5) M -Marker 100 bp ladder; 5a- Sequence contains *Eco*RV recognition site; 5b- Sequence not contain *Eco*RV recognition site

Figure 3

[See figure]