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Fall Armyworm *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) in East Nusa Tenggara Province of Indonesia: Genetic Characterization and Strain Detection

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Abstract

Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae) is an invasive pest of corn plants spreading throughout the world, including East Nusa Tenggara (NTT), Indonesia. Despite the wide distribution, there is a lack of information on the strain or genetic diversity of the pest in NTT. Therefore, this study aimed to determine the strain of *S. frugiperda* from several areas in NTT with a molecular method using *cytochrome oxidase subunit 1* (*COI*) and *triose phosphate isomerase* (*Tpi*) gene markers. The samples were collected from 3 islands: Timor, Flores, and Sumba. Amplification of the marker genes was carried out using 3 specific primers to identify the strain obtained from samples. Subsequently, polymerase chain reaction (PCR) products were sequenced and the DNA sequences were analyzed using the BioEdit and BLAST programs. Phylogeny analyses were carried out using the MEGA 11 program to verify the strain group of samples with reference isolates from other countries found in GenBank. The PCR results showed product amplicon size 811 bp for the *CO1A* marker. Based on phylogeny tree analysis using *COI* marker, *S. frugiperda* from NTT showed 2 clades, namely corn and rice strains. The characterization results showed that *S. frugiperda* in NTT comprised 63.6% corn and 36.4% rice strains. *COIB* classified *S. frugiperda* from NTT into the h4 haplotype subgroup, while *Tpi* gene marker was in the corn strain. This study provided valuable information regarding the strain of *S. frugiperda* in Indonesia to determine the appropriate control strategy.

Keywords: *COI*; genetic diversity; NTT; *Spodoptera frugiperda*; *Tpi*

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INTRODUCTION

The armyworm *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is an invasive pest native to the American continent (Nagoshi et al., 2017). In 2016, this pest was reported as an invasive pest in Central and West Africa (Goergen et al., 2016), which entered into India and Thailand in 2018 (Sun et al., 2021). The spread was reported in West Sumatra in 2019 (Sartiami et al., 2020) and East Flores Regency, East Nusa Tenggara (Nusa Tenggara Timur/NTT) in 2020 (Mukkun et al., 2021).

S. frugiperda damages plants by eating leaf tissue from 1 side, leaving only the epidermis. It also attacks the growing point, preventing the formation of shoots or young leaves at all stages (Shylesha et al., 2018; Trisyono et al., 2019). *S. frugiperda* can fly to approximately 100 km per day, showing distinctive characteristics

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such as a short life cycle, high adaptability, fecundity, non-diapausing (Wan et al., 2021), and a host range of 353 plant species from 76 families (Montezano et al., 2018). Based on estimations, damage due to pest attacks causes yield losses of between 15 to 73%, ranging from 8.3 to 20.6 million tons per year with an economic loss value of 2.5 to 6.2 billion per year (Day at al., 2017; Assefa, 2019). Wu et al. (2021) reported that the spread of *S. frugiperda* had an impact on the economic potential of agricultural-producing areas in China. This was based on the total potential cost (TPC) of 4 provinces including Yunnan (830.51 M$), Guangxi (346.09 M$), Sichuan (116.87 M$), and Shandong (116.43 M$) of more than 100 M$. In Indonesia, the pest attack caused damage to corn crops in Lampung ranging from 26.50 to 70% (Lestari et al., 2020), Bali 47.84% (Supartha et al., 2021), NTT 85 to 100%, and Ende Regency 34 to 76% (Pu'u and Mutiara, 2021). Management of *S. frugiperda* by land cultivation, early planting in the dry season, resistant corn cultivars, seed treatment, cropping system arrangement, as well as cultural, chemical, non-chemical, biological, and biotechnological methods have been proven effective for application in various countries to support sustainable agriculture (Kumar et al., 2022).

According to Wan et al. (2021), 2 strains of *S. frugiperda* (Strain R and C) attack various countries and continents. Specifically, strain R invades rice and grasses, while strain C attacks corn, cotton, and sorghum. These 2 strains are morphologically identical but differ in host range, mating behavior, genetic, and pheromone components (Dumas et al., 2015; De Groote et al., 2020). *S. frugiperda* also has genetic diversity and biotypes that are physiologically different. This genetic diversity is affected by strains through geographical spread factors (Monnerat et al., 2006). The 2 strains of fall armyworm (FAW) are identified mainly based on polymorphisms in the mitochondrial gene *cytochrome oxidase subunit 1* (*COI*) and the nuclear gene *triosephosphate isomerase* (*Tpi*) (Levy et al., 2002; Nagoshi, 2010). The mitochondrial *COI* is a popular DNA barcoding marker to identify species of Lepidoptera with accuracy. Furthermore, *Tpi* has been used to identify the host strains of FAW (Nagoshi et al., 2012; Jing et al., 2020). The results of molecular identification have shown that there are strains C and R in Africa, strain R in India, strain C in the Democratic Republic of the Congo, and strain C in China (Swamy et al., 2018; Assefa, 2019; Zhou et al., 2021; Malekera et al., 2023). In Indonesia, various types of *S. frugiperda* strains are distributed in some provinces, including rice, corn, and their combination which can be found in Banten (Sartiami et al., 2020), Lampung (Lestari et al., 2020), Sumatra (Nelly et al., 2021), respectively.

NTT is a central area for corn production used as a staple food and animal feed. However, the attack of *S. frugiperda* on corn covered 10,563 ha in February 2020, where approximately 213,899.62 ha of plants were threatened in several regencies. This phenomenon poses a significant risk to NTT region consisting of large and small islands and is known to affect the reduction of corn production to 859,230 tons. Previous studies have found that boat movements between islands are the entry route for *S. frugiperda* from one region to another (Sands et al., 1993). Consequently, the corn and rice strains of this pest when spread in NTT can cause damage to other important host plants. To overcome the potential distribution, knowledge of the types of strain and genetic diversity of the pest is very important for developing sustainable monitoring and management strategies (Swamy et al., 2018). However, there is no information on genetic diversity or strains of *S. frugiperda* in NTT, showing the need for further exploration. Therefore, this study aimed to identify the genetic diversity of *S. frugiperda* from NTT using molecular markers based on *COI* and *Tpi* genes. The results were expected to serve as a reference for decision-making on control methods of corn plantations in NTT in an integrated pattern supporting sustainable agriculture programs.

MATERIALS AND METHOD

Collection of *S. frugiperda*

S. frugiperda larvae were randomly collected on corn plantations from Kotabaru, Maukaro, Detusoko, Wolowaru, Ende Timur, Rindi, Temu, Ndapayami, Fatukanutu, Pukdale, Tuatuka in NTT Province, Indonesia, between March to July 2023. Samples were obtained from infested corn plant, which shows the symptoms of whorl and leaf defoliation and frass-filled damaged whorl. The larvae consisting of 3rd to 5th instar stadia were collected and put into bottles containing 70% ethanol (v/v) (Widarti et al., 2022), and labeled. Then single larvae from each location were identified molecularly at the Plant Protection Laboratory of the IPB University.

DNA extraction

DNA extraction was carried out using gSYNC DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) by weighing 0.05 caterpillars and placing them in a mortar with the addition of liquid nitrogen, followed by grinding with a pestle. After the powder form was obtained, 200 µl GST buffer with 20 µl proteinase k was added and transferred to a 1.5 ml tube for vortex. Incubation was performed at 60 °C for 1 hour and every 10 minutes the tube was inverted to achieve a clear sample. During incubation, the elution buffer was heated for each sample of 200 µl. Subsequently, centrifugation was conducted at 14,000 rpm for 2 minutes and the supernatant was transferred to a new 1.5 ml tube, added with 200 µl GSB buffer, and shaken quickly for 10 seconds (Sorvall Biofuge Fresco, USA). This was followed by the addition of 200 µl absolute ethanol, which was also shaken quickly for 10 seconds. All samples were transferred to the GS column and centrifuged at 14,000 rpm for 1 minute. GS column is a spin column made of silica to bind the DNA in the DNA purification process. The GS column was further transferred to a new 2 ml collection tube, added with 400 µl of W1 buffer, and centrifuged at 14,000 rpm for 1 minute. The supernatant was discarded and returned to the GS column in a 2 m collection tube. Finally, 600 µl of wash buffer added with absolute ethanol was centrifuged at 14,000 rpm for 1 minute.

DNA amplification

In this study, a total of 2 primers were used, namely *CO1* and *Tpi*. The primers were purchased from Integrated DNA Technologies, Inc., USA, and were ordered through Genetika Science Indonesia, Inc. The barcode area from *CO1* used primers 101F and 911R to amplify a DNA fragment with a size of 811 bp. To obtain the subgroup haplotype of *CO1*, the primers used were 893F and 1303R to achieve a 410 bp fragment (Nagoshi et al., 2007). Meanwhile, *Tpi* amplification was carried out using primers 282F and 850R to produce a 500 bp fragment (Nagoshi, 2010; Nagoshi et al., 2017) (Table 1).

Polymerase chain reaction (PCR) reactions were performed using Dream Taq® Green Mastermix 2X. DreamTaq® Green Mastermix (2X) (Thermo Fisher Scientific USA, Catalog Number K1081) is a ready-to-use PCR master mix that contains all the necessary components for PCR amplification, except for the template DNA and primers. This master mix was ordered through Genetika Science Indonesia Inc. with a standard buffer. Specifically, PCR was conditioned with 5 initial denaturations at 94 °C for 1 minute, followed by 33 cycles (denaturation at 92 °C for 30 seconds, annealing at 56 °C for 30 seconds, and elongation at 72 °C for 45 seconds), and final elongation at 72 °C for 3 minutes (Nagoshi et al., 2017).

Electrophoresis

Electrophoresis was performed to test the quality of PCR results by mixing 10 µl sample with 6 µl of gel loading buffer. All samples including 100 bp DNA ladder were run with 1.8% agarose gel containing GelRed in 0.5X Tris-borate buffer (TBE 45 mM, Tris base 45 mM, boric acid, 1 mM EDTA pH 9.0), following the procedure of Nagoshi et al. (2017). The results of electrophoresis were visualized using a UV transilluminator and photographed with a digital camera. Meanwhile, PCR results containing *S. frugiperda* DNA along with the primers were sent to a commercial sequencing company for nucleotide sequencing (Tamura et al., 2021).

Sequencing

PCR products were qualitatively analyzed using 1% agarose gel electrophoresis and samples were sent to Apical Scientific Sdn. Bhd. (Malaysia) for sequencing. Nucleotide sequence data were compiled using BioEdit software and analyzed with the Basic Local Alignment Search Tool (BLAST) program (www.ncbi.nlm.nih.gov). The phylogenetic relationships were constructed using Molecular Evolutionary Genetic Analysis (MEGA) v 11.0 software with 1,000 bootstrap replicates (Tamura et al., 2021).

Table 1. Primers for identifying host-specific strains and haplotypes of *S. frugiperda* on corn

Primer name	Primer forward and reverse	References
COIA	101F 5'-TTCGAGCTGAATTAGGGACTC-3' 911R 5'-GATGTAAAATATGC TCGTGT-3'	Nagoshi et al. (2007)
COIB	893F 5'-CACGAGCATATTTTACATCWGCA-3' 1303R 5'-CAGGATAGTCAGAATATCGACG-3'	Nagoshi et al. (2007)
Tpi 282F	282F 5'-GGTGAAATCTCCCCTGCTATG-3'	Nagoshi (2010); Nagoshi et al. (2017)
Tpi 850R	850R 5'-AATTTTATTACCTGCTGTGG-3'	

Characterization of *COI* and *Tpi* gene segments

S. frugiperda strains were identified based on 3 amplified target genes, namely the *CO1A*, *CO1B*, and *Tpi* genes. Sequence analysis of the *CO1A* gene was used to identify *S. frugiperda* species. The *CO1B* gene was used to identify haplotypes based on the regions consisting of *C-h1*, *C-h2*, *C-h3*, and *C-h4* (Nagoshi et al., 2007; Nagoshi et al., 2017). The results of haplotype acquisition were calculated using the formula $= \frac{h4-h2}{h4+h2}$, to determine the type of strain, namely FAW[TX], FAW[FL], or FAW[M] (mix). The FAW[TX] strain is shown by a calculation result of ≤ -0.3, while the FAW[FL] was ≥ 0.1. The FAW[M] group has a calculation index value of $0.3 < x < 0.1$, which is a combination of the 2 previous groups (Nagoshi et al., 2017). *Tpi* gene sequence analysis was carried out to identify strains based on host type consisting of *Tpi-C* (corn), *Tpi-R* (rice), and *Tpi-H* (corn and rice transition). Haplotypes within *Tpi* were observed based on the gTpi183Y polymorphism in exon-4. Strains *Tpi-C*, *Tpi-R*, and *Tpi-H* were characterized by the base sequence in exon 4 respectively, namely C, T, and Y (C/T).

Data analysis

Genetic diversity data based on *COIA* gene sequences were analyzed sequentially. The process involved editing the sequence result with BioEdit, aligning them using Clustal W within the BioEdit program, reconstructing the phylogenetic tree using the neighbor-joining method, and performing 1,000 bootstrap replicates with MEGA XI. The sequences were compared with authentic sequences alongside outgroup sequences as controls obtained from the GenBank database. A similar process was carried out to analyze *COIB* and *Tpi* gene sequences.

RESULTS AND DISCUSSION

Characterization of *S. frugiperda* in NTT using *COIA*

The results of the *COI* marker from samples 1 (Flores-Kotabaru), 2 (Detusoko), 3 (East Ende), 4 (Maukaro), and 5 (Wolowaru) amplified using primer pairs 101F and 911R obtained a DNA band with a size of approximately 810 bp, as shown in Figure 1. This success indicated that the amplification method used was effective in isolating *COI* gene from samples originating from various regions.

The phylogenetic tree of *COI* shows 2 clades of *S. frugiperda*, namely corn and rice strains, as presented in Figure 1 and Figure 2. Samples from NTT were grouped with corn and rice strains from Florida (HM136586 and HM136593). Meanwhile, corn strains were found in Pukdale, Wolowaru, Rindi, Maukaro, Temu, Flores (Kotabaru), and Tuatuka. Meanwhile, rice strains were found in Fatukanutu, East Ende, Detusoko, and Ndapayami.

Characterization of *S. frugiperda* in NTT using *COIB*

All corn strains showed the h4 haplotype based on sites 1164 (G) and 1287 (G), as presented in Table 2. Calculation using the formula $= \frac{CSh4-CSh2}{CSh4+CSh2}$ produced an index value of more than 0.1, showing that *S. frugiperda* had a FAW[FL] haplotype profile. Therefore, the haplotype profile of *S. frugiperda* in NTT was close to those

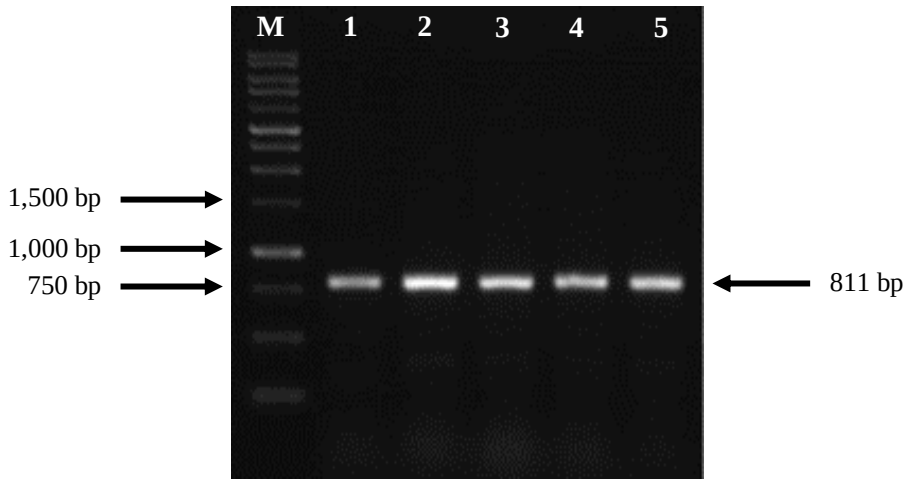

Figure 1. Visualization of PCR results of *COI* marker (a) from samples 1 (Flores-Kotabaru), 2 (Detusoko), 3 (East Ende), 4 (Maukaro), and 5 (Wolowaru) which are amplified using primer pairs 101F and 911R with a DNA fragment measuring 811 bp

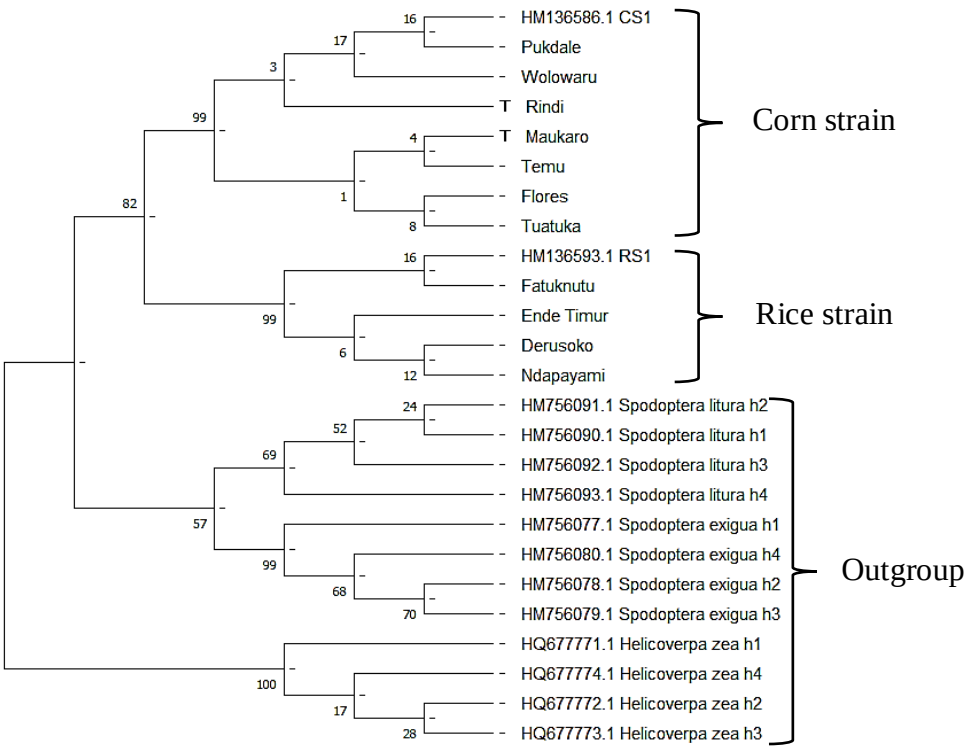

Figure 2. Phylogenetic tree based on the *COIA* gene using the Maximum Likelihood method and 1,000x bootstrap showing the clustering of 2 *S. frugiperda* strains from NTT

in Florida, which was widely found in Africa (Nagoshi et al., 2017; Nagoshi et al., 2019). The haplotype profile in Southeast Asia has only been known in Myanmar, as also reported by similar studies conducted in India and Africa (Nagoshi et al., 2020).

The phylogenetic analysis of *COI* gene sequences showed the existence of 2 distinct clades within the *S. frugiperda* populations, corresponding to the well-known corn and rice strain of this species, as shown in Figure 1 and Figure 2. This distinction was significant because the strains were associated with different host plant preferences, which could impact pest management strategies. The samples collected from the NTT were found to cluster with both corn and rice strains, identified in Florida (GenBank accession numbers HM136586 and HM136593), showing a possible introduction or migration across geographical regions.

Individuals identified as belonging to the corn strain were located in several areas within NTT, including Pukdale, Wolowaru, Rindi, Maukaro, Temu, Flores (Kotabaru), and Tuatuka. This distribution suggested a wide prevalence of corn strains in these agricultural zones, which could correlate with the dominant cultivation of maize. Rice strain was predominantly found in Fatukanutu, East Ende, Detusoko, and Ndapayami. This spatial distribution showed the ecological adaptation of rice strain to regions where cultivation was more prevalent, indicating host specificity. The presence of both strains within close geographic proximity in NTT underscored the complex dynamics of *S. frugiperda* populations and increased important considerations for local pest control measures, as different strains showed varying responses to management practices.

Characterization of *S. frugiperda* in NTT using *Tpi*

Based on characterization using *Tpi*, all samples found in NTT were corn strains or *Tpi-C*, as shown in Table 3. Identification of this strain was based on the Tpi183Y site of exon 4 (gTpi183Y), which showed C183. Specifically, the characteristics of *Tpi-C* were found to have 2 variants based on sites 192 and 198, as observed in samples in Africa, namely AfrCa1 (*Tpi-Ca1*) and AfrCa2 (*Tpi-Ca2*). The locations of Flores (Kotabaru), East Ende, Maukaro, Wolowaru, Rindi, Ndapayami, Futukanutu, and Pukdale were closer to AfrCa1 (C192 and C198), while Detusoko, Temu, and Tuatuka were closer to AfrCa2 (T192 and T198).

Table 2. Polymorphisms showing haplotype in the *S. frugiperda* maize strain (*COI-CS*) at various locations in NTT in *COIB* area

Code	Nucleotide position				Location	References
	1122	1125	1164	1287		
JN573287.1 (h1)	C	T	A	A	Florida, USA	Nagoshi et al. (2007)
JN573288.1 (h2)	-	-	-	G	Florida, USA	Nagoshi et al. (2007)
JN573289.1 (h3)	-	-	G	-	Florida, USA	Nagoshi et al. (2007)
JN573290.1 (h4)	-	-	G	G	Florida, USA	Nagoshi et al. (2007)
Flores	-	-	G	G	Indonesia, Flores (121°58'52.5" E and 8°32'22.4" S)	
Detusoko	-	-	G	G	Indonesia, Detusoko (121°44'42.39" E and 8°43'44.44" S)	
Ende Timur	-	-	G	G	Indonesia, Ende Timur (121°40'44.92" E and 8°49'31.80" S)	
Maukaro	-	-	G	G	Indonesia, Maukaro (121°31'40.73" E and 8°37'15.98" S)	
Wolowaru	-	-	G	G	Indonesia, Wolowaru (121°52'29.95" E and 8°47'44.68" S)	
Rindi	-	-	G	G	Indonesia, Rindi (120°37'57.97" E and 10°03'48.98" S)	
Temu	-	-	G	G	Indonesia, Temu (120°14'17.57" E and 9°38'07.90" S)	
Ndapayami	-	-	G	G	Indonesia, Ndapayami (120°07'05.89" E and 9°38'38.14" S)	
Fatukanutu	-	-	G	G	Indonesia, Fatukanutu (123°53'40.44" E and 10°10'04.56" S)	
Pukdale	-	-	G	G	Indonesia, Pukdale (123°49'43.30" E and 10°07'40.28" S)	
Tuatuka	-	-	G	G	Indonesia, Tuatuka (123°50'30.10" E and 10°09'11.46" S)	
AfrCsa1	-	-	G	-	Africa	Nagoshi et al. (2019)
AfrCsa2	-	-	-	-	Africa	Nagoshi et al. (2019)
COI-RS consensus	-	C	-	-	Western Hemisphere	Nagoshi et al. (2019)
COI-CS consensus	-	-	R*	R*	Western Hemisphere	Nagoshi et al. (2019)

Note: R = A/G

In the analysis of *S. frugiperda* populations in NTT, all corn strains were identified as possessing the h4 haplotype. This identification was based on the presence of specific nucleotide sites at positions 1164 and 1287 of the *COI* gene, characterized by the *nucleotide guanine* (G). The sites played a significant role in differentiating between haplotypes within the species, representing specific genetic markers that distinguished the h4 haplotype from others.

The significance of nucleotide sites was in their role as molecular signatures that allowed for the classification and tracking of different haplotypes of *S. frugiperda* across various geographic regions. In this case, the presence of guanine at both 1164 and 1287 positions

Table 3. Polymorphisms showing haplotypes in *S. frugiperda* corn strains in various locations in NTT in the *Tpi* area

Code	Nucleotide position (exon 4)								Locations
	129	144	165	168	180	183	192	198	
GQ411914.1 (*Tpi-C*)	C	G	C	T	C	C	T	T	USA[1]
AfrCa1 (*Tpi-C*)	-	-	-	-	-	-	C	C	Africa[2]
AfrCa2 (*Tpi-C*)	-	-	-	-	-	-	-	-	Africa[2]
Flores	-	-	-	-	-	-	C	C	Flores (121°58'52.46" E and 8°32'22.35" S)
Detusoko	-	-	-	-	-	-	-	-	Detusoko (121°44'42.39" E and 8°43'44.44" S)
Ende Timur	-	-	-	-	-	-	C	C	Ende Timur (121°40'44.92" E and 8°49'31.80" S)
Maukaro	-	-	-	-	-	-	C	C	Maukaro (121°31'40.73" E and 8°37'15.98" S)
Wolowaru	-	-	-	-	-	-	C	C	Wolowaru (121°52'29.95" E and 8°47'44.68" S)
Rindi	-	-	-	-	-	-	C	C	Rindi (120°37'57.97" E and 10°03'48.98" S)
Temu	-	-	-	-	-	-	-	-	Temu (120°14'17.57" E and 9°38'07.90" S)
Ndapayami	-	-	-	-	-	-	C	C	Ndapayami (120°07'05.89" E and 9°38'38.14" S)
Fatukanutu	-	-	-	-	-	-	C	C	Fatukanutu (123°53'40.44" E and 10°10'04.56" S)
Pukdale	-	-	-	-	-	-	C	C	Pukdale (123°49'43.30" E and 10°07'40.28" S)
Tuatuka	-	-	-	-	-	-	-	-	Tuatuka (123°50'30.10" E and 10°09'11.46" S)
AfrRa1 (*Tpi-R*)	T	A	-	C	G	T	C	C	Africa[2]
AfrCa1/Ra1 (*Tpi-H*)	Y*	R*	-	-	-	Y*	C	C	Africa[2]
AfrCa2/Ra1 (*Tpi-H*)	Y*	R*	-	-	-	Y*	Y*	Y*	Africa[2]
MT767446.1 (*Tpi-R*)	T	A	-	C	G	T	C	C	Tiongkok
Tpi-R consensus	-	-	T	C	-	T	-	-	Western Hemisphere[2]
Tpi-C consensus	-	-	-	-	-	-	Y*	Y*	Eastern Hemisphere[2]

Note: Y = C/T, R = A/G; [1]Nagoshi (2010); [2] Nagoshi et al. (2019)

confirmed the h4 haplotype, which was associated with the corn strain of *S. frugiperda*. The genetic consistency among corn strains in NTT suggested a relatively uniform population in terms of haplotype.

The differentiation between haplotypes played a significant role in understanding the evolutionary history, migration pattern, and adaptation of *S. frugiperda*. The calculation using the formula $=\frac{CSh4-CSh2}{CSh4+CSh2}$, which obtained an index value greater than 0.1, further supported the classification of these populations as having the FAW[FL] haplotype profile. Moreover, this profile was correlated with populations found in Florida and Africa, showing possible historical connections or similar environmental pressures influencing the populations.

The identification of the FAW[FL] haplotype in NTT was particularly significant because it suggested that the haplotype profile of *S. frugiperda* was similar to Florida and widely distributed in Africa, as reported by Nagoshi et al. (2017). This correlation showed the potential for global dispersal of the pest and adaptability to different environments.

The reference to Southeast Asia, particularly Myanmar, where similar haplotype profiles were observed, suggested a broader regional pattern. The similarity in haplotype profiles between

NTT and other regions, such as India and Africa, according to Nagoshi et al. (2020), showed the widespread nature of the pest and the ability to maintain specific genetic traits across diverse geographic locations. Understanding these haplotype distributions was essential for developing targeted pest management strategies that considered the genetic diversity and adaptability of *S. frugiperda* populations.

Based on the phylogenetic tree, *S. frugiperda* from NTT had the closest kinship to the samples in Florida (Figure 3). The characterization results showed that *S. frugiperda* originating from several locations in NTT were classified into 2 strains, namely rice and corn. The classification was based on the *COIA* gene marker consisting of 63.6% corn samples from Pukdale, Wolowaru, Rindi, Maukaro, Temu, Flores (Kotabaru), and Tuatuka as well as 36.4% rice samples from Fatukanutu, East Ende, Detusoko, and Ndapayami. Similarly, Dharmayanthi et al. (2022); Fahmi et al. (2023); and Nagoshi et al. (2020) reported that the proportion of rice strains in Southeast Asia based on *COI* gene marker was relatively dominant. However, Zhang et al. (2024) stated that *S. frugiperda* was identified as a corn strain according to *COI* and *Tpi* gene markers. This was because corn varieties provided higher efficiency in nutrient conversion and assimilation as well as adaptability in the digestion and detoxification process by increasing amylase and lipase activity (Zhang et al., 2024). The proportion of corn strains was attributed to the availability of wider land in NTT, ranging from 280 to 309 ha in 2020 to 2022 (Statistics of Nusa Tenggara Timur Province, 2022; 2023). Meanwhile, rice fields are only around 170 to 180 ha in the same year. This variation in land area contributed to the greater development of corn strains compared to rice. *COIA* is part of the *COI* gene segment from the 5' direction, which contains information in the form of barcodes and host strain markers (Nagoshi et al., 2017; Nagoshi et al., 2020).

Regarding the characterization based on *COIB*, *S. frugiperda* from NTT was included in the subgroup of the h4 haplotype. This showed that NTT samples were close to Florida because of the higher proportion of *CS-h4* (Nagoshi et al., 2017; Nagoshi et al., 2018; Nagoshi et al., 2020). Similarly, Nagoshi et al. (2020) stated the dominant haplotype found in Southeast Asia was h4. *COIB* is part of the *COI* gene segment from the 3' direction, which contains information regarding the subgroup (Nagoshi et al., 2017; Nagoshi et al., 2020).

Based on the *Tpi* gene marker, all samples were classified as corn strains with 2 variants, namely AfrCa1 (*Tpi-Ca1*) and AfrCa2 (*Tpi-Ca2*) in line with sites 192 and 198. In this study, *Tpi-H* was not similar to the sample observed in Myanmar by Nagoshi et al. (2020). This can be the basis for the assumption that rice strains are absent in NTT. Therefore, interstrain mating has not occurred to produce hybrid strains based on the *Tpi* gene marker (*Tpi-H*). The presence of the *Tpi-H* strain in Southeast Asia requires significant consideration due to the possibility of spreading to surrounding communities in Indonesia. Consequently, there is a need for periodic monitoring as a form of early detection of strains that threaten.

Strain detection using 2 gene markers yielded a homogeneous combination (*COI-CS Tpi-CS*) However, Fahmi et al. (2023) conducted an experiment in Bogor and found a hybrid *S. frugiperda* strain (*COI-RS Tpi-CS*) based on *COI* and *Tpi* gene markers. Although this study showed a homogeneous strain combination, hybrid strains remain possible in Indonesia. This inconsistency of detection with *COI* and *Tpi* gene markers could be attributed to the suitability of the gene markers. Based on the results, *COI* was found to be more suitable in the West compared to Indonesia which is in Southeast Asia. Meanwhile, *Tpi* was considered appropriate for use in the Eastern and is more accurate in showing the main feed preferences of *S. frugiperda* (Nagoshi et al., 2020). This was supported by the consistency of detection results with *Tpi*, which showed good corn strains in Myanmar, Bogor, and NTT.

The identification of the h4 haplotype in corn strains of *S. frugiperda* in NTT showed the need for region-specific pest control strategies that were informed by the genetic makeup of local pest populations. Due to the homogeneous combination of *COI-CS* and *Tpi-CS* markers found in this study, *S. frugiperda* population in NTT was relatively uniform in genetic composition, which could be advantageous for developing targeted pest management methods. However, the discovery of hybrid strains such as the *COI-RS Tpi-CS* strain in other parts of Indonesia, namely Bogor, indicated that hybridization could still occur, potentially leading to more complex pest dynamics. This showed the need for continuous genetic monitoring

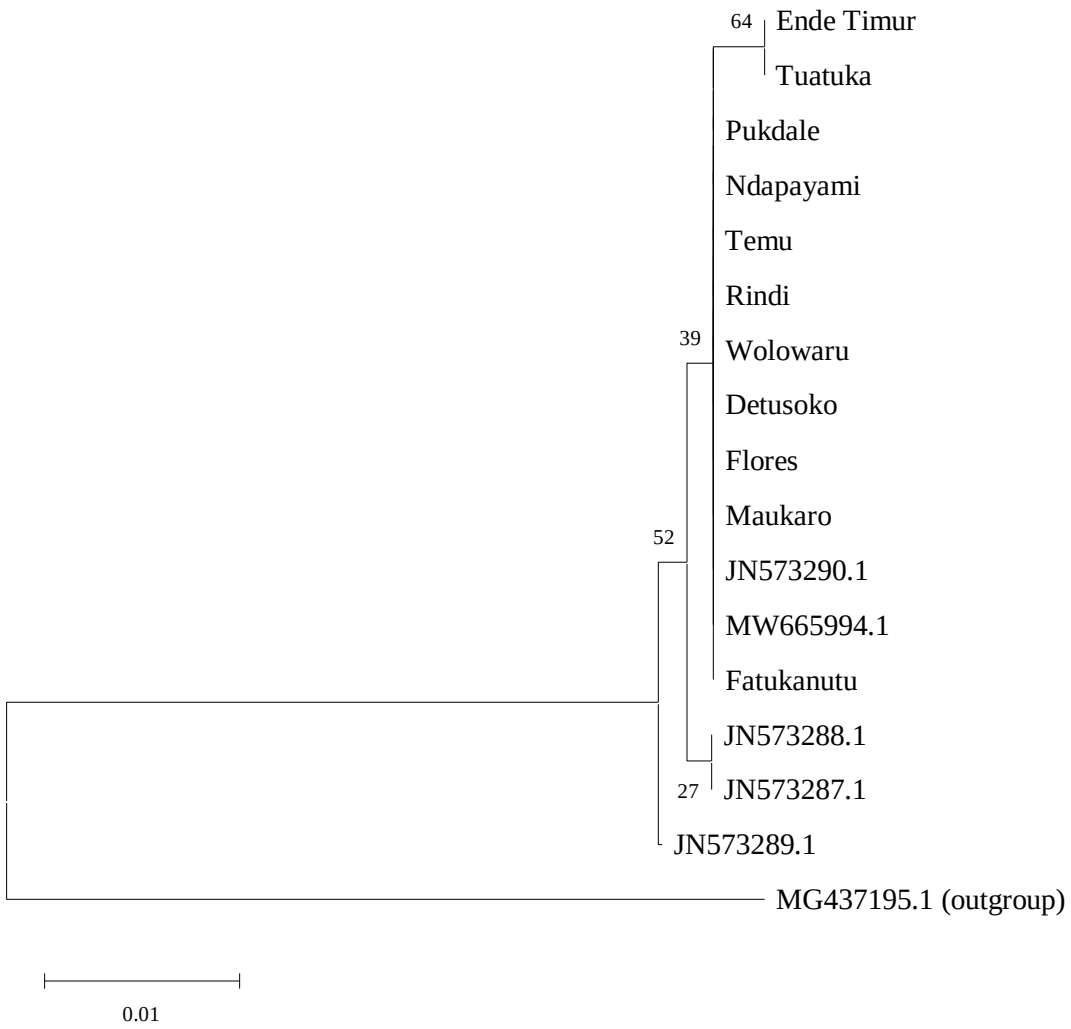

Figure 3. Phylogeny tree of *S. frugiperda* based on nucleotide sequences of the *CO1B* gene processed using the Neighbor-Joining method with the Kimura-2 model (1,000× bootstrap)

in NTT to detect any changes in the population structure, including the occurrence of hybrid strains, which required different or additional control measures.

The regional differences in the effectiveness of gene markers, with *COI* being more suitable in western regions and *Tpi* proving accurate in eastern regions, such as NTT, suggested that pest management practices must be tailored to local genetic characteristics. The *Tpi* marker reliability in detecting the corn strain in NTT correlated with the region agricultural focus on maize. These results should guide the selection of control strategies that were specifically effective against the h4 haplotype. For instance, pest control measures might include the use of maize varieties resistant to the specific characteristics of the h4 haplotype or the timing of interventions to target the life cycle stages most vulnerable to disruption. Additionally, understanding these genetic distinctions could help avoid the deployment of broad-spectrum methods that might be less effective or inadvertently promote the spread of hybrid or resistant strains.

CONCLUSIONS

This study identified and confirmed the presence of both corn and rice strains of *S. frugiperda*, marking the 1st report in NTT. The genetic diversity of the pest in corn plants, based on the *COI* marker, revealed *COI-CS* and *COI-RS* strains, both belonging to the h4 haplotype. Genetic diversity based on the *Tpi* marker identified the *Tpi-C* strain, indicating the corn strain. Futher analysis is recommended to verify the presence of rice and corn strains across all cor-producing regions in NTT.

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