



## Genetic variation of pest fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) in different landscapes in Bogor

Keragaman genetik hama ulat gerayak jagung *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) pada lanskap yang berbeda di Bogor

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### ABSTRACT

*Spodoptera frugiperda* is an invasive pest from the American continent that attacks corn (*Zea mays*) and rapidly invaded Africa and Asia. Two main factors that support migration and population distribution of this species are suitable habitats and human activities. To date, two genetic strains of *S. frugiperda* have been found in corn in Indonesia: the corn strain (CS) and the rice strain (RS). The most accurate gene markers to detect these strains are *COI* and *Tpi*, which are located in mitochondria and Z chromosome. This study aims to determine the existing strains of *S. frugiperda* and their distribution in various landscapes in Bogor Regency. The research was conducted from July 2020 to December 2021 in Bogor, West Java. Sampling of *S. frugiperda* was carried out from corn plants in Leuwisadeng, Pamijahan1, Pamijahan2, Kemang, Tenjolaya, Dramaga, Cigombong, Cijeruk, Tamansari, and Ciomas. Larval samples were collected and preserved using 96% ethanol, followed by DNA extraction, DNA amplification, electrophoresis, and DNA sequencing. Distribution data were analyzed using QGIS and Google Earth Pro programs, and statistical analysis was performed using SPSS 22. Sequence data were edited using GeneStudio, aligned using ClustalW in BioEdit, and the phylogeny tree was reconstructed using the neighbor-joining method (bootstrap 1000x) using MEGA X. The obtained sequences were compared with sequences from the GenBank® database. The results showed the presence of two distinct strains of *COI* (*COI-CS*4 and *COI-RS*) and one strain of *Tpi* (*Tpi-C*) in Bogor. The study found no relationship between the landscape structure and genetic variation of *S. frugiperda*.

**Key words:** *COI*, invasive species, landscape, *Tpi*

### ABSTRAK

*Spodoptera frugiperda* merupakan hama invasif asal benua Amerika yang menyerang tanaman jagung (*Zea mays*) dan menginvasi Afrika hingga Asia pada tahun 2016. Dua faktor utama yang mendukung migrasi dan distribusi populasi dari spesies ini adalah kesesuaian habitat dan aktivitas manusia. Keragaman genetik *S. frugiperda* berdasarkan *host strain* terdiri atas strain jagung (CS) dan strain padi (RS) yang keduanya dapat ditemukan di pertanaman jagung. Di Indonesia, strain tersebut telah ditemukan di Lampung (CS) dan Banten (RS). Gen penanda yang paling akurat untuk mendeteksi strain ini adalah *COI* dan *Tpi*, yang masing-masing terletak di mitokondria dan kromosom-Z. Penelitian ini bertujuan untuk mengetahui strain *S. frugiperda* yang ada dan sebarannya di berbagai lanskap di Kabupaten Bogor. Penelitian dilaksanakan pada bulan Juli 2020 hingga Desember 2021

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di Kabupaten Bogor, Jawa Barat. Pengambilan sampel dilakukan dengan pencuplikan larva *S. frugiperda* dari tanaman jagung di Leuwisadeng, Pamijahan1, Pamijahan2, Kemang, Tenjolaya, Dramaga, Cigombong, Cijeruk, Tamansari, dan Ciomas. Sampel larva kemudian dikoleksi dan diawetkan menggunakan alkohol 96% dan kemudian dilakukan ekstraksi DNA. Data distribusi dianalisis menggunakan aplikasi QGIS dan Google Earth Pro dengan SPSS 22 untuk analisis statistik. Data sekuen diedit menggunakan GeneStudio, *aligning* menggunakan ClustalW di BioEdit, dan rekonstruksi pohon filogeni menggunakan metode *neighbour-joining* (bootstrap 1000x) pada aplikasi MEGA X. Sekuen yang didapat kemudian dibandingkan dengan sekuen dari database GenBank®. Hasil penelitian menunjukkan keberadaan dari dua strain yang berbeda dari *COI* (*COI*-CSh4 dan *COI*-RS) dan satu strain *Tpi* (*Tpi*-C) di Bogor. Penelitian ini juga menemukan bahwa struktur lanskap tidak memiliki hubungan dengan variasi dari *S. frugiperda*.

**Kata kunci:** *COI*, spesies invasif, lanskap, *Tpi*

## INTRODUCTION

The fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) is an invasive pest of corn (*Zea mays*) originating from the American continent (FAO 2020). In 2016, *S. frugiperda* was reported to invade corn crops in western and central Africa (Goergen et al. 2016) and the following year in India, China, Myanmar, and Thailand (FAO 2020). In 2019, this insect invaded corn plants in Indonesia, including North Sumatra (Girsang et al. 2020), West Sumatra, Banten, West Java (Bogor) (Sartiami et al. 2020), Lampung (Trisyono et al. 2019; Lestari et al. 2020), as well as Garut, Bandung, and Sumedang areas (Maharani et al. 2019). Its large reproductive capacity, absence of diapause, and wide host range (353 plants from 76 families) contribute to its rapid growth and invasion in areas with corn cultivation (Goergen et al. 2016; Montezano et al. 2018).

Environmental factors that significantly impact the migration and distribution pattern of *S. frugiperda* are habitats with suitable climates. *S. frugiperda* will stop and settle in habitats with suitable climates. During migration and population dispersal, genetic mixing can also occur, resulting in genetic variation in a location (Nagoshi et al. 2019). Human activities also affect the distribution of *S. frugiperda* by facilitating the movement of plant material from one place to another (Wang et al. 2020).

The genetic variation of *S. frugiperda* based on the host consists of the corn strain (CS) and the rice strain (RS) (Pashley 1986; Jacobs et al. 2018). These strains can be detected using *COI* and *Tpi* (*Triose phosphate isomerase*). In *Tpi*, the strains are represented by *Tpi*-R (rice strain), *Tpi*-C

(corn strain), and *Tpi*-H (*Tpi*-C/*Tpi*-R). *Tpi*-H is a heterozygous on the male sex chromosome (ZZ), which can produce *Tpi*-C and *Tpi*-R on different Z chromosomes (Nagoshi 2010). *COI* area provides information about DNA barcodes, strains, and haplotypes that distinguish between two geographically separated populations. *Tpi* area provides information about the strain on gTpi183Y in exon-4 (Nagoshi et al. 2019). Combining information from the *COI* and *Tpi* areas is very helpful in determining the population origin of *S. frugiperda* at a particular location.

The corn strain (CS) variation of *COI* is divided into four subgroups based on sites 1164 and 1287. These subgroups are described as CS-h1 (A[1164] A[1287]), CS-h2 [A G], CS-h3 [G A], and CS-h4 [G G] (Nagoshi et al. 2007). Based on these subgroups, the maize strain (*COI*-CS) is classified into three haplotype profiles, namely FAW[TX], FAW[FL], and FAW[M]. FAW [TX] is a haplotype profile of a population with the highest CS-h2 ratio, referring to the profile of a population originating from Texas (USA). FAW [FL] is the haplotype profile that has the most CS-h4 and refers to the population profile originating from Florida (USA). FAW [M] is a combination of the two profiles (Nagoshi et al. 2008; Nagoshi et al. 2015; Nagoshi et al. 2017b). This haplotype profile is stable and useful in studying the long-distance movements of *S. frugiperda* (Nagoshi et al. 2008; Nagoshi et al. 2015; Nagoshi et al. 2017b).

Land configuration is one factor that influences the genetic population structure of species. Landscape spatial and dynamic configurations are essential to the genetic processes that construct gene variation within species (Holderegger & Wagner 2006). A landscape can affect the

distribution pattern of a particular genotype that appears only in suitable habitats. The suitable habitat acts as a corridor, while the unsuitable habitat acts as a barrier. This corridor promotes the dispersal process of an insect genotype, generating genetic similarity in a location (Holzhauer et al. 2006; Malaquias et al. 2020). A study on *Ostrinia furnacalis* (Guenée) demonstrated the relationship between genes and the landscape in insects. The study found that mitochondrial haplotype H12 has a positive correlation with corn crops and a negative correlation with other crops such as vegetables, oilseed crops, and cotton. Haplotype H12 tends to be present in locations with corn crops and absent in locations with other crops. Thus, there is an association between the appearance of haplotype H12 and corn crops (Dong et al. 2021).

As a new invasive pest in Indonesia, research on the geographical distribution of *S. frugiperda* needs to be carried out. Information about the distribution and genetic variation of *S. frugiperda*

is required for control purposes. It is also necessary to determine the origin of *S. frugiperda* and its existing variants. Currently, it is unknown which strains have entered Indonesia, including Bogor, which has also been invaded by *S. frugiperda* in corn. Furthermore, it is essential to examine the habitat landscape that supports specific genetic variants of *S. frugiperda*. Therefore, the aim of this research is to study the geographical distribution and genetic variation of *S. frugiperda* in Bogor Regency, West Java.

## MATERIAL AND METHOD

### Sample collection

Sampling of *S. frugiperda* was conducted in ten invested corn fields in Bogor Regency (Figure 1; Table 1). DNA isolation, amplification, and electrophoresis were carried out at the Insect Pathology Laboratory, Department of Plant

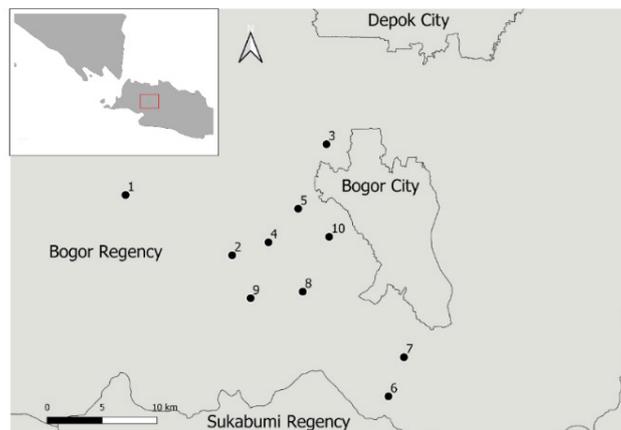


Figure 1. Sampling sites in Bogor Regency.

Table 1. Sampling locations of *Spodoptera frugiperda* in Bogor

Location (District)	Code	Date	Elevation (m asl)	Corn field area (m <sup>2</sup> )	Coordinate	
					Latitude	Longitude
Leuwisadeng	1	10 August 2020	225	940	6°34'11.0"S	106°34'53.5"E
Pamijahan 2	2	22 July 2020	350	1.250	6°37'09.7"S	106°40'09.7"E
Kemang	3	21 July 2020	153	4.500	6°31'37.8"S	106°44'47.7"E
Tenjolaya	4	20 July 2020	313	4.000	6°36'30.7"S	106°41'57.0"E
Dramaga	5	17 July 2020	194	500	6°34'50.3"S	106°43'24.6"E
Cigombong	6	14 July 2020	517	2.000	6°44'08.6"S	106°47'53.7"E
Cijeruk	7	30 August 2020	457	2.500	6°42'11.8"S	106°48'39.3"E
Tamansari	8	27 July 2020	582	880	6°38'57.5"S	106°43'39.4"E
Pamijahan 1	9	24 August 2020	595	6.000	6°39'17.4"S	106°41'04.8"E
Ciomas	10	25 August 2020	234	2.300	6°36'13.8"S	106°44'56.6"E

Protection, IPB University. The research was conducted from July 2020 to December 2021. Larval samples were taken from each location and put into 96% ethanol. The measured environmental parameters included elevation and the description of the location landscape within a radius of 300 m.

### DNA extraction

DNA was extracted from ten *S. frugiperda* larvae using a modified Doyle and Doyle method (Doyle & Doyle 1990). About 50-60 mg of larval body parts were put into a 1.5 ml tube along with 400 µl of 65 °C CTAB buffer solution (2% CTAB, 50 mM Tris-HCl 0.1 M, 0.02 M EDTA, 1.4 M NaCl, in Mercaptoethanol 1%). Larvae and buffer solution were crushed using a plastic micropestle. The crushed larvae were then vortexed for 10 seconds and incubated in a water bath at 60 °C for 30 minutes. The sample was added with a mixture of chloroform: isoamyl alcohol (CI) 24:1 60 µl and vortexed for 10 seconds. The mixture was then centrifuged at 10,000 rpm for 3 minutes. The supernatant formed was transferred to a new tube. The DNA solution was then added with isopropanol at a temperature of -20 °C, as much as 0.7 of the total volume of the supernatant. The solution was centrifuged for 3 minutes at 10,000 rpm, then the liquid formed was removed with a micropipette. The pellets were then washed twice with 500 µl of 70% ethanol each and allowed to dry (the tube was inverted) for 12 hours on filter paper at room temperature. Each DNA pellet was dissolved in 100 µl TE. The DNA extraction samples were incubated at 37 °C for 1 hour and stored in the refrigerator at -20°C.

### DNA amplification, electrophoresis, and sequencing

Amplification of *COI* segment was done with two kinds of primers, *COIA* and *COIB*. *COIA* primers are 101F (5'-TTCGAGCTGAATTAGGGACTC-3') and 911R (5'-GATGTAAAAATA TGCTCGTGT-3') to produce an 811 bp fragment. *COIB* primers are 893F (5'-CA CGAGCATATTTTACATCWGCA -3') and 1303R (5'- CAGGATAGTCAGAAT ATCGACG -3') to obtain a 410 bp fragment (Nagoshi et al. 2007). Meanwhile, *Tpi* amplification was done with primers (5'- GGTGAAAT

CTCCCCTGCTATG -3') and 850R (5'- AATTTTATTACCTGCTGTGG -3') to produce 500 bp fragments (Nagoshi 2010; Nagoshi et al. 2017a). PCR reactions were performed using the MyTaq™ HS RedMix with standard buffer. PCR was conditioned with an initial denaturation of 94 °C for 1 min, followed by 33 cycles (denaturation at 92 °C for 30 s; annealing 56 °C for 30 s; and elongation at 72 °C for 45 s), and final elongation at 72 °C for 3 min (Nagoshi et al. 2017a). All samples and a 100 bp DNA ladder were separated on a 1.0% agarose gel containing RedSafe™ Nucleic Acid Staining Solution 20,000x (2 µl) in 0.5X Tris-Acetate-EDTA (TAE) buffer. Electrophoresis results were visualized using a UV transilluminator. The PCR results containing *S. frugiperda* DNA along with the primers were sequenced by a third-party company.

### Data analysis

DNA sequence data were edited using GeneStudio, aligned using ClustalW in BioEdit, and used to reconstruct the phylogeny tree using the neighbor-joining method (bootstrap 1000x) in MEGA X. The sequences obtained were compared with sequences from the GenBank® database. Distribution data were analyzed using QGIS and Google Earth Pro, and SPSS 22 for statistical analysis (t-test). The haplotype profile of the corn strain was calculated using the formula  $(CSh4 - CSh2)/(CSh4 + CSh2)$ . FAW [TX] has an index value  $\leq -0.3$ ; FAW [FL]  $\geq 0.1$ ; and FAW [M]  $-0.3 < x < 0.1$  (Nagoshi et al. 2017b).

## RESULTS

### Characterization of FAW in Bogor using *Cytochrome Oxidase Subunit I (COI)*

The phylogenetic tree of *COI* reveals two clades of *S. frugiperda*, corn strain (CS) and rice strain (RS) (Figure 2). The sequence samples from Bogor cluster with corn and rice strains from Florida (HM136586 and HM136593); Three samples of the corn strain (*COI*-CS) and seven samples of the rice strain (*COI*-RS). The corn strains are found in Leuwisadeng, Kemang, and Cigombong. The rice strains are found in Pamijahan 2, Tenjolaya, Dramaga, Cijeruk,

Tamansari, Pamijahan 1, and Ciomas. These corn strain samples are all categorized as the subgroup of haplotype h4 or CS-h4 (Table 2). This means that sites 1164 and 1287 show guanine (G).

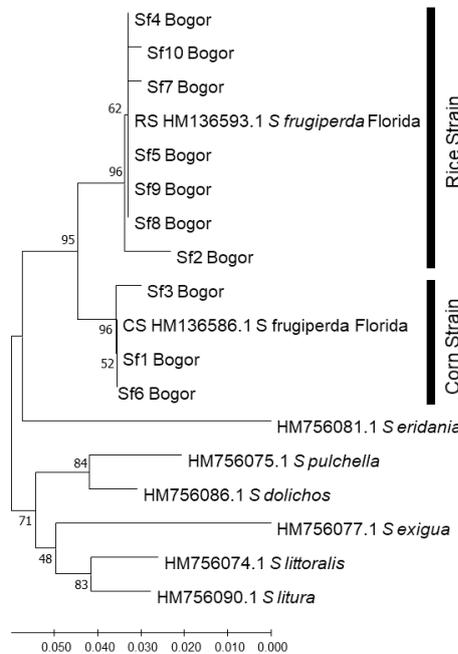
Ciomas were characterized as AfrCa1. Pamijahan 2, Kemang, and Tenjolaya were characterized as AfrCa2 (Table 3).

**Characterization of FAW in Bogor using *Triosephosphate Isomerase (Tpi)***

Based on site Tpi183Y of exon 4 (gTpi183Y), all samples were classified as C<sub>183</sub>. This means that all samples found in Bogor Regency were corn strains or *Tpi-C* (Table 3). Based on sites 192 and 198 of exon 4, the characteristics of *Tpi-C* in Bogor Regency are AfrCa1 and AfrCa2. AfrCa1 has C at sites 192 and 198 (C<sub>192</sub> and C<sub>198</sub>). AfrCa2 has T on sites 192 and 198. Leuwisadeng, Dramaga, Cigombong, Cijeruk, Tamansari, Pamijahan 1, and

**Landscape structure and genetic variation**

The landscape structure around corn fields in Bogor Regency consisted of roads, rivers/waters, settlements, trees, paddy fields, fields, and abandoned/vacant land (Figure 3). The field is a class that has the largest area of the landscape. However, fields in Bogor were not uniformly planted within a 300 m radius. Cornfields accounted for only about 0.25 ha out of more than 10 ha of fields. Each farmer in Bogor Regency had a relatively narrow land area, and they grew crops that were spatially and temporally diverse.



**Figure 2.** Phylogeny tree based on *Cytochrome Oxidase I (COI)* gene with *neighbor-joining* method and *bootstrap* 1000x that showed two group of strain. The Bogor sequences were submitted on GenBank (Accession Number: ON753769-ON753778).

**Table 2.** Polymorphism sites that show subgroup haplotype in corn strain FAW based on *COIB*

Code	Nucleotide site				Location	Reference
	1122	1125	1164	1287		
JN573287.1 (h1)	C	T	A	A	USA	Nagoshi et al. 2007
JN573288.1 (h2)	-	-	-	G	USA	Nagoshi et al. 2007
JN573289.1 (h3)	-	-	G	-	USA	Nagoshi et al. 2007
JN573290.1 (h4)	-	-	G	G	USA	Nagoshi et al. 2007
1,3,6	-	-	G	G	Bogor, Indonesia	This study
AfrCsa1	-	-	G	-	Afrika	Nagoshi et al. 2019
AfrCsa2	-	-	-	-	Afrika	Nagoshi et al. 2019

The settlements had the highest number of patches (NumP), which means they have scattered fragments (up to more than 27 patches/location). The trees did have a relatively large area but were quite fragmented because of the high NumP value (Table 4). The altitude of the land varied from 153 m asl to 595 m asl (Table 1).

Ten landscape variables were statistically analyzed using a t-test based on the *COI* variation (corn and rice strains). The analysis results did not show a significant effect at the 5% level of the ten landscape variables tested (Table 4).

## DISCUSSION

The phylogenetic tree of *COI-A* showed that *S. frugiperda* in Bogor clustered into two clades, *COI-CS* and *COI-RS*. 70% (7/10) of the samples were *COI-RS*, and 30% (3/10) were *COI-CS*. This result is similar to the genetic variation of *S. frugiperda* in several locations in Indonesia that took samples in 2019 (Dharmayanthi et al. 2022) and Southeast Asia that were predominated by *COI-RS* (Nagoshi et al. 2020). Those studies indicate that the *COI-RS* is uniformly predominant in various geographical areas.

All the corn strains in the Bogor Regency are categorized as h4 (*COI-CS-h4*) and can be classified as FAW [FL]. It means the haplotype profile of *S. frugiperda* in Bogor Regency is close to *S. frugiperda* in Great Antille and Florida (Nagoshi et al. 2017a; Nagoshi et al. 2018; Nagoshi et al. 2020). This haplotype profile in Southeast Asia has only been discovered in Myanmar with FAW [FL] and is similar to profiles in India and African countries (Nagoshi et al. 2020). In Indonesia, this profile has never been studied before.

Based on *Tpi*, all samples in this study showed the corn strain (*Tpi-C*). Recent studies on *Tpi* in *S. frugiperda* in Indonesia (Dharmayanthi et al. 2022) and Myanmar (Nagoshi et al. 2020) also showed similar results. The difference in the results of these two countries is the presence of *Tpi-H*. It means that Myanmar had rice strain in the form of *Tpi-H* (*Tpi-R/Tpi-C*). Therefore, *Tpi-H* can be in Indonesia at any time. Early detection of *Tpi-H* and *Tpi-R* in Indonesia is necessary because these strains have the potential to invade paddy fields (Nagoshi et al. 2020).

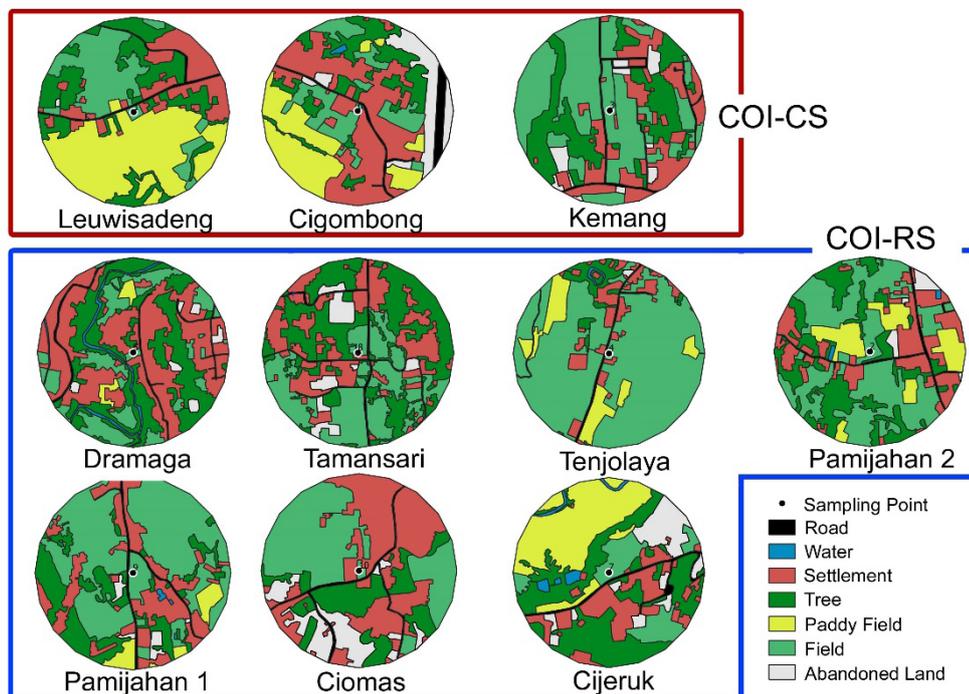
There are two types of *S. frugiperda* in this study, *COI-RS Tpi-C*, and *COI-CS Tpi-C*. The characterization of *Tpi* and *COI* in this study resulted from the same individual. It means one individual can have rice strain from *COI* (*COI-RS*) and corn strain from *Tpi* marker (*Tpi-C*). The presence of a discordant strain (*COI-RS Tpi-C*) in an individual *S. frugiperda* is influenced by the intermating of a female rice strain and a male corn strain (Nagoshi 2010; Nagoshi et al. 2020). Most of the *S. frugiperda* population in Indonesia consisted of the *COI-RS Tpi-C* strain (Dharmayanthi et al. 2022), similar to populations in China, India, and Africa. However, existing populations in those countries suggest that *S. frugiperda* was introduced in small numbers from the Western Hemisphere or its natural habitat. The small numbers are believed to have come exclusively from corn crops in America. A small portion (about 20%) of those living in the corn crops are *COI-RS*. This strain and other corn strains of *S. frugiperda* invaded Africa and Asia and were detected exclusively in corn. That is why *Tpi* in the eastern hemisphere is more accurate in indicating host-associated strains, while *COI* in the western hemisphere is more informative (Nagoshi et al. 2020).

The distribution of *S. frugiperda* strains in Bogor Regency based on *COI* indicates that corn strains are found in locations on the outskirts, such as Cigombong, Leuwisadeng, and Kemang (Figure 1; Figure 2). Meanwhile, rice strains were found in the middle of Bogor. The locations where the rice strain of *COI* was found had different landscape conditions. Tenjolaya (code 4), which has a large agricultural field, can have the same strain as Dramaga (code 5), where the sampling location is in the middle of settlements (Table 1; Figure 3). This can also be observed in Pamijahan 1 (code 9), where the highest location exhibits the same strain as low location, such as Dramaga (code 5) and Ciomas (code 10). The result of the t-test also shows no significant difference in the landscape variable. Thus, this study found no significant landscape differences between corn and rice strains (Table 4). There are no visible barriers; only a corridor is found due to the presence of corn crops. However, the corridor could not distinguish between the presence of the two *COI* strains. Thus, this finding supports the idea that the corn strain of *S. frugiperda* in Asia and Africa represents a small

**Table 3.** Polymorphism sites that show strains FAW based on *Tpi*

Code	Nucleotide site (exon 4)								Location
	129	144	165	168	180	183	192	198	
GQ411914.1 ( <i>Tpi</i> -C)	C	G	C	T	C	C	T	T	USA <sup>1</sup>
AfrCa1 ( <i>Tpi</i> -C)	-	-	-	-	-	-	C	C	Africa <sup>2</sup>
AfrCa2 ( <i>Tpi</i> -C)	-	-	-	-	-	-	-	-	Africa <sup>2</sup>
1,5,6,7,8,9,10	-	-	-	-	-	-	C	C	Bogor
2,3,4	-	-	-	-	-	-	-	-	Bogor
Consensus <i>Tpi</i> -R	-	-	T	C	-	T	-	-	Western Hemisphere <sup>2</sup>
Consensus <i>Tpi</i> -C	-	-	-	-	-	-	Y*	Y*	Western Hemisphere <sup>2</sup>

\*Y= C/T; <sup>1</sup>Nagoshi 2010; <sup>2</sup> Nagoshi et al. 2019.



**Figure 3.** Landscape map in 300 m radius from sampling point in Bogor Regency that grouped by *COI*-CS and *COI*-RS.

**Table 4.** Statistical analysis (t-test) of landscape variables based on *COI* corn (n = 3) and rice (n = 7) strain

Variable	Corn strain (Means ± SD)	Rice strain (Means ± SD)	P-value
CA Trees (ha)	5.97 ± 1.27	6.50 ± 3.04	0.78
NumP Trees	11.00 ± 2.65	18.29 ± 8.48	0.20
CA Settlements (ha)	6.68 ± 2.44	6.73 ± 3.17	0.98
NumP Settlements	20.00 ± 8.54	27.00 ± 6.66	0.20
CA Fields (ha)	7.50 ± 4.59	10.38 ± 6.07	0.49
NumP Fields	9.67 ± 6.35	8.57 ± 5.16	0.78
CA Paddy fields (ha)	5.48 ± 5.34	2.20 ± 2.70	0.22
NumP Paddy fields	2.67 ± 2.52	3.14 ± 2.48	0.79
Elevation (m asl)	298.33 ± 192.76	389.29 ± 160.03	0.46
Corn field area (m <sup>2</sup> )	2480.00 ± 1827.90	2490.00 ± 1946.20	0.99

CA: class area; NumP: number of patch.

fraction compared to the western hemisphere, where it is primarily found in corn crops than paddy fields (Nagoshi et al. 2020). *Tpi* was not statistically tested in this study because all samples exhibited *Tpi*-C, indicating that landscape variables had no significant influence on *Tpi* variation, except for the presence of corn crops. Further investigation into landscape and host strain is necessary, involving additional locations, samples, and a broader radius.

## CONCLUSION

The genetic variation of *S. frugiperda* in corn fields in Bogor, as determined by *COI* analysis, consisted of three samples of *COI*-CS and seven samples of *COI*-RS. All *COI*-CS samples have h4 haplotypes and can be classified as FAW [FL] profile haplotypes. Based on *Tpi* analysis, all ten samples exhibit the *Tpi*-C strain. Geographically, *COI*-CS is predominantly found in the outskirts of Bogor Regency, while *COI*-RS is primarily found in the central area of Bogor Regency. The variation in the landscape within a 300 m radius in Bogor Regency does not correlate with the variation in host strains of *S. frugiperda*, based on *COI* and *Tpi*.

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