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Article

Spatial Distribution and Genetic Diversity of The Fall Armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) in the Bali Province and Lesser Sunda, Indonesia

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Abstract: *Spodoptera frugiperda* is an invasive pest that has spread in various parts of the world. These pests have the ability to spread and adapt highly to new habitats. Until now, it is not known with certainty the distribution of these invasive pests in Eastern Indonesia, especially Bali and Nusa Tenggara. This study aims to map the spatial distribution and genetic distribution of *S. frugiperda* which damages maize in the areas of Bali and Nusa Tenggara. This research was conducted using a survey method from May to September 2022 covering the islands of Bali, Lombok, Sumbawa, Sumba, Flores, and Timor. The results showed that *S. frugiperda* had spread evenly in Bali and Nusa Tenggara. The results of PCR amplification in the COI gene from 9 sample isolates from all research locations showed the similarity of DNA bands leading to the *Spodoptera frugiperda* species with a banding pattern length of 683 – 697. These results indicated that the distribution of genetic variants of corn caterpillars in Bali, NTB, and NTT was confirmed as *S. frugiperda* species. However, the isolated gene *S. frugiferda*, which was shown by the alignment results of the sequences from Lombok, was confirmed as a different strain from strains from Bali, Sumba, Sumbawa, Flores, and Timor. This incident can be seen from the difference in the protein base composition of *S. frugiperda* from Bali, Sumba, Sumbawa, Timor, and Flores. The results of phylogenetic analysis in this study confirmed 3 clusters of the genetic closeness of *S. frugiperda*. Cluster-1 comes from the results of the search for specimens of JB FAW and KB FAW from Bali, SB FAW and SB FAW Sorghum from Sumba, SW FAW from Sumbawa, KP FAW from Timor, and FL FAW from Flores. Cluster-2 is an isolate outside of our species. Cluster-3 comes from the search for LT and LS FAW specimens from Lombok. The genetic distance between cluster-1 and cluster-3 is quite far, which is 0.20 mu.

Keywords: The Fall Armyworm, *Spodoptera frugiperda*, invasive species, mapping distribution, DNA-barcoding

1. Introduction

The Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is one of the invasive pests and is responsible for decreasing crop yields and causing economic losses in the agricultural sector in the world [1]. These pests damage agricultural crops such as corn, rice, sorghum, wheat, barley, buckwheat, oats, ryegrass, soybeans, tobacco, tomatoes, potatoes, peanuts, cotton, sugar beets, alfalfa, and onions and more than 300 other plant species [2]. As a well-known pest, FAW can produce yield losses ranging from

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15 – 73% with the most preferred host plant being corn. FAW is reported to have experienced rapid growth in several countries in America, Africa, and Asia [3,4]. The larvae are capable of causing a yield loss of 10.6% in maize in northeastern Mexico [5]. In some more serious cases such as in Honduras, Central America, maize yields were reduced by 40% [6]. FAW populations were also reported to have invaded maize crops in Ghana and Zambia with loss percentages of 26.6% and 35%, respectively [7].

In 2016, for the first time FAW was reported to have successfully invaded maize crops in West and Central Africa (Benin, Nigeria, Sao Tome, Prin-cipe and Tongo) and then spread to all countries on the African continent [8]. In 2018, this pest was reported in India and first attacked maize plantations in the Karnataka area. Based on this incident, many attribute the rapid adaptability to the advantage for these insect pests to invade quickly into various areas [9]. The potential spread of FAW can be influenced by climate, expansion of host plants, to agricultural commodity trading activities [10,11].

In Indonesia, this pest was first reported in 2019, precisely in corn plantations in Sumatra [3] In Indonesia, this pest was first reported in 2019, precisely in corn plantations in Sumatra [12]. Reports on the results of the study reported that FAW attacked maize plantations in the Bali Province and reduced yields quite high in the lowlands [13,14].

Recognizing the early signs of the presence of a pest to be able to overcome this problem is very important to do. Based on this, apart from understanding the defining features of insect species diversity are basic ecological and biological traits, errors in making accurate taxonomic identification are important issues in biological research that allow the application of adequate measures to be able to implement appropriate control measures [15–18]. Errors in the identification process can lead to ineffective control measures that have the potential to increase the impact caused by certain pest species including FAW [19–21]. Biodiversity studies, systematics, community ecology and bio-monitoring are also highly dependent on proper species identification activities [22,23]. The standard method of utilizing morphological characters becomes a challenge due to various factors such as phenotypic variations, geographical location, eating habits, and epigenetic influences [24–26].

In the last three decades, mitochondrial DNA has been extensively studied and proved to be an important tool in species delimitation because it has biological properties and is ideal as a molecular marker of biodiversity [27,28]. This molecular approach is to use DNA Barcoding which allows rapid processing of the young instar phase, as well as the fragmented body parts of the cuticle [3]. Partial DNA sequences of mitochondrial genes such as Cyto-chrome oxidase I (COI) and other molecular markers have been used for the discovery and discovery of new species. COI fragments measuring about 648 bp can be used as DNA barcodes to identify and differentiate animal species in several zoological studies [29].

DNA Barcoding is a new field of technology and molecular classification has been applied to plant protection science [30,31]. The application of this technique is very useful to be able to detect early the possibility of an invasive pest attack in an area [32]. Research applying species based on classical taxonomy is often misleading with some species being misdefined based on their association with the host plant.

The use of DNA Barcoding in monitoring the invasion of FAW pests has previously been reported that the molecular characteristics of FAW in rice and maize lines in Sumatra Province indicate that the presence of FAW in South Sumatra is close to FAW isolates from corn strains from Lampung and West Sumatra [3]. The findings of FAW have also been reported successfully with morphological and molecular techniques on several host plants in eighteen districts of Bhutan [33]. The larvae that were found in corn fields in Lampung were FAW with no nucleotide differences in the COI gene sequence between FAW isolates in Lampung Province and FAW isolates from abroad [34].

Based on this, the importance of the world economy and reports of failures in agricultural productivity due to FAW insects may be an important component in the development of IPM plans to be able to suppress the attack of this pest. We designed distribution maps and molecular markers to detect the level of spatial distribution and genetic diversity of FAW on various host plants in Bali and Sunda Leser Provinces. This is done

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because the nature of FAW has the potential to spread to various host plants through adaptation to their habitat and feed needs. The provinces of Bali and Nusa Tenggara are two provinces located in the Sunda Leser with the main agricultural commodities being corn, rice and sorghum. Based on this, the purpose of this study was to determine the map of the spatial distribution and genetic diversity of FAW in the province of Bali – Nusa Tenggara which attacks agricultural crops from the Poaceae family. The results of this study are considered very important to be used as information and insight to relevant authorities, farmers, and academics in preparing follow-up plans in controlling FAW attacks.

2. Materials and Methods

Study area and sample collection

The research was conducted at the field and laboratory scale from May - September 2022. The field-scale investigation consisted of determining the sampling location and sampling. Integrated Pest Control Laboratory (IPMLab), Faculty of Agriculture, Udayana University, Bali, conducts laboratory-scale research, including specimen preservation and pest identification. The spelling takes place at the Corn Plantation Center in Bali and the Lesser Sunda Islands. Purposive sampling was used to collect samples of maize plants in Bali, Flores, Kupang, Lombok, Sumba and Sumbawa (Table 1). On each island, three to 10 locations of maize crops were taken which showed damage due to FAW attacks.

Table 1. Sampling locations

Island	Regency	Locations	Place Altitude (Masl)	Coordinate point
Bali	Badung	Plaga	965	8018'22" S 115013'50" E
		Abiansemal	187	8032'11" S 115013'24" E
	Tabanan	Marga	331	8027'44" S 11509'51" E
		Selemadeg	143	8031'21" S 11502'45" E
	Jembrana	Mendoyo	54	8024'3" S 114046'48" E
		Melaya	45	8014'54" S 114028'36" E
	Buleleng	Grokgak	57	8010'56" S 114045'49" E
		Kubutambahan	1328	8013'15" S 115013'20" E
	Karangasem	Karangasem	77	8027'55" S 115037'15" E
		Sidemen	219	8030'43" S 115025'14" E
	Klungkung	Klungkung	79	8033'35" S 115023'58" E
	Gianyar	Blahbatuh	89	8035'23" S 115018'13" E
	Denpasar	Denpasar Timur	18	8039'28" S 115015'24" E
	Bangli	Kintamani	1253	8014'31" S 115016'31" E
Lombok	Lombok Tengah	Pujut	12	8053'15" S 116020'13" E
		Batukliang	383	803720" S 116019'41" E
	Lombok Barat	Labuapi	27	8038'52" S 11606'56" E
		Sekotong	15	8046'47" S 11602'52" E
		Narmada	179	8035'38" S 116013'54 E
	Lombok Utara	Kayangan	21	8016'0" S 116014'55" E
	Lombok Timur	Terara	329	8038'25" S 116024'25" E
		Pringgabaya	5	8038'18" S 116025'18" E

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		Sambelia	94	8022'48" S 116041'47" E
		Jerowaru	161	8046'41" S 11602'53" E
	Mataram	Gerung	43	8037'6" S 11607'36" E
Sumba	Sumba Barat Daya	Wewewa Timur	463	9030'46" S 119016'2" E
	Sumba Timur	Kambera	38	9040'27" S 119018'9" E
Sumbawa	Sumbawa	Moyohilir	31	8035'30" S 117031'11" E
		Plampang	58	8044'6" S 117044'8" E
		Labuan Badas	7	8026'50" S 117019'50" E
		Buer	19	8027'24" S 11702'43" E
	Sumbawa Barat	Jereweh	13	8050'4" S 116049'9" E
		Seteluk	47	8039'33" S 116051'1" E
		Poto Tano	41	8035'27" S 116050'30" E
Kupang	Kupang	Taebenu	319	10013'56" S 123042'27" E
		Sulamu	28	1001'5" S 123044'53" E
		Kupang Timur	20	1006'38" S 123049'8" E
	Belu	Atambua	332	906'3" S 124053'33" E
	Malaka	Laenmanen	342	9021'58" S 124049'24" E
	Timor Tengah Utara	Insana	466	9025'35" S 124044'37" E
Flores	Ende	Ndona	25	8050'43" S 121040'48" E
		Ende Timur	43	8049'42" S 121040'35" E
		lokoboko	55	8049'55" S 121040'49" E

***Distribution of* FAW**

The population of FAW was determined and recorded based on the sample location. Furthermore, the distribution of FAW in Bali Province was determined by documenting the sample locations and the emergence of FAW from that place. Bali, Flores, Kupang, Lombok, Sumba and Sumbawa Island are the sample locations in this study. The data obtained were then placed into a thematic map using the Q-GIS 3.16.16 program.

Genetic diversity of FAW

DNA extractions

DNA extraction was carried out according to the methods given by Hamid et al. [35] and Supartha et al. [36]. In summary, adult specimens found within the study site was preserved in 70% alcohol and stored in a -20°C freezer until the material was required for isolation. The sample must be isolated, collected, and dried on a towel for 30 minutes. The larvae were then submerged in 85°C hot water for 30 minutes, until they became a yellowish colour. The locations in the two abdominal sections were then cut and put in a 1.5 µL tube. Proteinase K was added in the quantity of 5 µL and crushed until crushed. The crushed material (pH 7.5) was dissolved in 300 µL of TNES buffer containing 1 M Tris HCl. ddH2O, and 20% SDS), homogenized, and incubated at 60°C for 3 hours. After the incubation time was fulfilled, 85 µL of 5 M NaCl was added and centrifuged for 10 minutes at 14000 rpm. The supernatant was collected in large amounts (up to 400 µL) and deposited in a new tube with isopropanol, up to 60% of the volume taken. Placed it in the freezer for 20 minutes after that. 5 minutes of centrifugation at 14,000 rpm. After removing the supernatant, 500 µL of cold 70% alcohol was added before centrifuging for 15 minutes

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at 14,000 rpm. The supernatant was extracted once again and dried at room temperature for 24 hours. After drying, 20 µL of TE buffer was added (1st Base, Malaysia). The DNA suspensions were stored at -20°C before being used.

DNA amplifications

Forward primer Lep F1 (5'-ATT CAA CCA ATC ATA AAG ATAT-3') and reverse primer Lep R1 (5'-TAA ACT TCT GGA TGT CCA AAAA-3') were used to amplify the Cytochrome Oxidase Subunit I (COI) region. The amplification technique was followed by Amouroux et al. [37]. In all, 5 µL of pH 8.3 PCR buffer (10 Mm Tris-HCl, pH 8.3; 1.5 Mm MgCl2; and 50 Mm KCl; 0.01% NP-40), 35 mL of distilled water, 200 mM dNTP, 1 unit of Taq Polymerase, 0.3 M primer, and 1-4 µL of DNA template were used in each PCR. PCR was carried out in stages, with one cycle running for one minute at 94°C, five cycles involving one minute at 94°C, 1.5 minutes at 45°C, 1.5 minutes at 72°C, 35 cycles taking one minute at 94°C, 1.5 minutes at 50°C, and one minute at 72°C, and the last cycle for five minutes at 72°C. The PCR reactions were then electrophoresed for 70 minutes at 55 V on an agarose gel with 1 µL of Ethidium Bromide (EtBr: 0 mg/mL/20 mL agarose). The data was shown using a UV transilluminator (UVP, USA).

Sequencing and phylogenetics tree analysis

The PCR findings were used to continue the sequencing procedure at 1st Base Malaysia. The sequencing data was examined at PT. Genetika Science Indonesia. Using the Bioedit Version 7.0.5.3 program, the base sequences of the two samples were evaluated in sequence to determine the difference in the base composition of the protein. The analytical findings were then sent to the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sample similarities and potential identities. To determine genetic distance, the MEGA program version 6.06 for Windows was utilized. The COI gene phylogenetic analysis was then performed using the Neighbor-Joining (NJ) technique with 1000 bootstrap replicates (Tamura-Nei model). The NCBI program (https://www.ncbi.nlm.nih.gov/) provided the reference strain utilized in this investigation [29,38].

3. Results

3.1. Spatial distribution of FAW in the Provinces of Bali and the Lesser Sunda

The spatial distribution shown by FAW in this study is evenly distributed in the provinces of Bali and the Lesser Sunda (West Nusa Tenggara and East Nusa Tenggara) (Figure 1). This even distribution can be triggered by the types of host plants planted by farmers in each region which is dominated by corn. Heterogenicity of habitat and topographical variations also provide opportunities for insect pests to invade.

3.2. The genetic diversity of FAW in the provinces of Bali and Lesser Sundas

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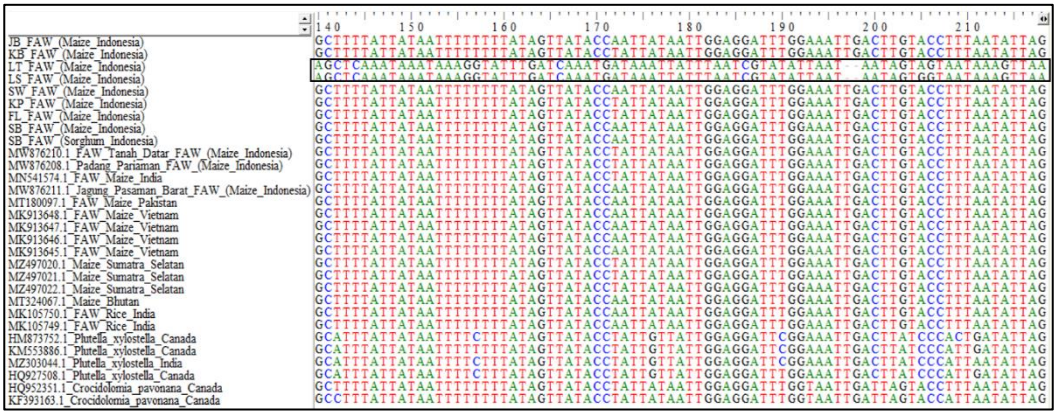
The results of DNA amplification using COI gene primers from 9 sample isolates obtained from this study showed consistent similarity of DNA bands with S. frugi-perda species, with band patterns at 683 – 697 bp (Figure 2).

Figure 2. Results of DNA amplification of FAW isolates using mitochondrial COI primers. M: Marker, (1) Jembrana, (2) Karangasem, (3) East Lombok, (4) South Lombok, (5) Sumbawa, (6) Kupang, (7) Flores, (8) Sumba and (9) Sorghum Sumba.



Likewise, the results of the alignment analysis showed that the genetic variants of FAW in the Lombok area were indicated to have different strains from other regions in this study such as Bali, Sumba, Sumbawa, Kupang, and Flores (Figure 3). Likewise, there was a deletion of nucleotide sequences in isolates LT_FAW (Maize Indonesia) and LS_FAW (Maize_Indonesia) precisely in sequence numbers 197 and 198 in this study. However, in general, these two types of FAW isolates have significant differences when compared to other isolates in this study.

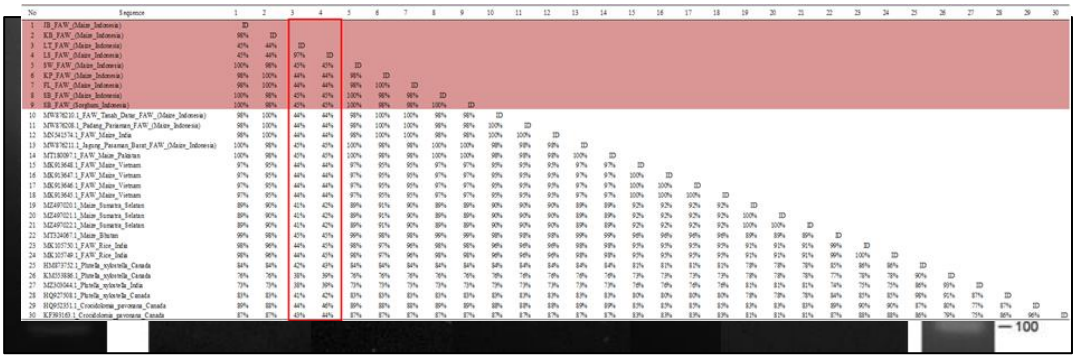


Figure 3. The results of the silenced sequences of FAW isolates obtained from the Bali province and Sunda Leser.

The results of the analysis of 9 sequences obtained, only sequences from East Lombok and South Lombok isolates had a low level of genetic similarity or <50%. This may indicate that there may have been a change in the genetic strain of FAW isolates obtained from the Lombok area, when compared to isolates obtained from other sampling locations (Figure 4).

Figure 4. The genetic similarity of FAW isolates obtained in this study and compared with isolates from several different countries. JB_FAW= Jembrana Bali, KB_FAW= Karangasem Bali, LT_FAW= East Lombok, LS_FAW= South Lombok, SW_FAW= Sumbawa, KP_FAW= Kupang, FL_FAW= Flores, SB_FAW= Sumbawa and SB_FAW Sorghum= Sumbawa Sorghum.

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The results of the phylogenetic analysis also showed that the FAW isolates obtained from this study were grouped into three clusters. The results of the search for isolates obtained from the province of Bali with codes JB_FAW and KB_FAW, isolates from Sumba (SB_FAW and SB_FAW Sorghum), Sumbawa (SW_FAW), Kupang (KP_FAW) and Flores region (FL_FAW) included one cluster with isolates from the Pariaman, Pasaman West, and Tanah Datar from Indonesia, Bhutan, Pakistan, Vietnam, India and 1 isolate from rice strains reported from India. Meanwhile, isolates obtained from the Lombok region (LT and LS_FAW) were included in the third group cluster (Figure 5).

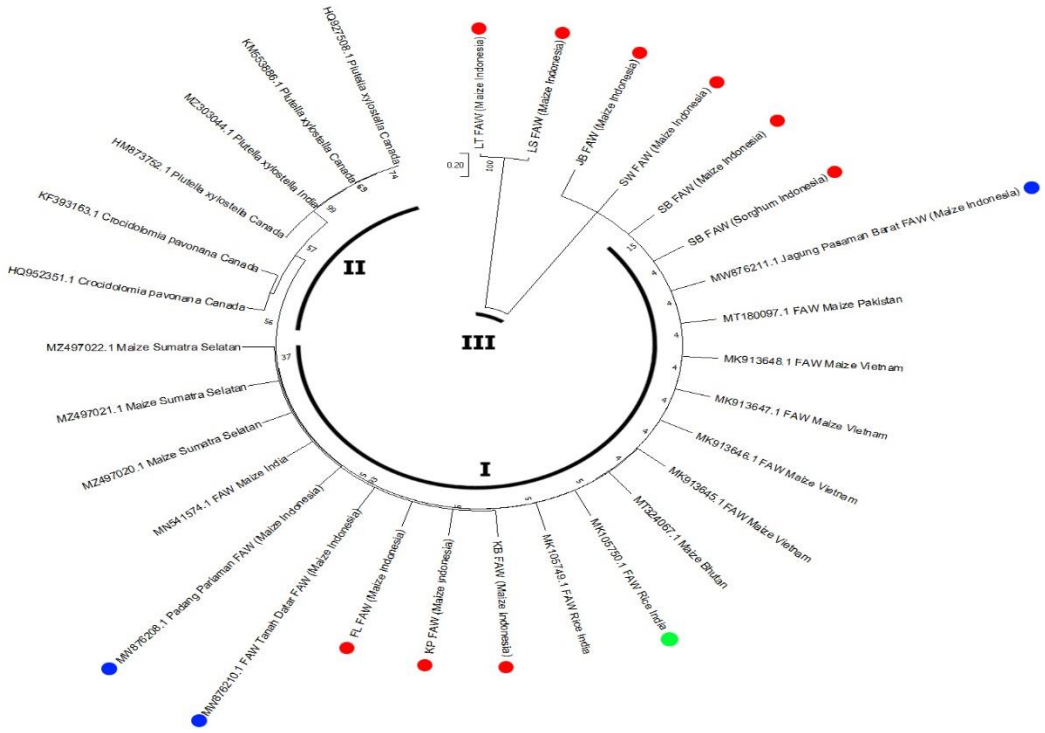

Figure 5. The phylogenetic tree was developed based on the COI gene using the Maximum Likelihood method (1000×; Tamura-Neimodel). *Spodoptera frugiperda* isolates collected from this study , Isolate from Indonesia , and Isolate Rice from India .

3.4. Conditions of maize cultivation in the provinces of Bali and Lesser Sunda as the main host of FAW

The results of the research in tracing the distribution location of FAWin the Bali-Nusra region, found several types of planting corn in each region. The availability of host plants that are always available in the area of Bali to Nusa Tenggara resulted in a very fast spread. Planting methods and land use in planting corn by local farmers have different characteristics in each region. Corn plantations in Bali are always available, because during the rainy season farmers plant corn in the highlands, and vice versa in the dry season farmers plant corn in the lowlands after harvesting lowland rice crops. These conditions are relatively the same as in the Lombok area. However, in the areas of Sumbawa, Sumba, Kupang and Flores, maize is the main commodity in these areas, so that the availability of maize as a host plant for FAW is always present in the areas of Bali and Nusa Tenggara. The condition of the corn fields planted on the islands of Bali and Lombok have the same land characteristics, namely corn cultivation is mostly carried out on former rice plantations or paddy fields. On the island of Sumbawa, however, corn is mostly grown under

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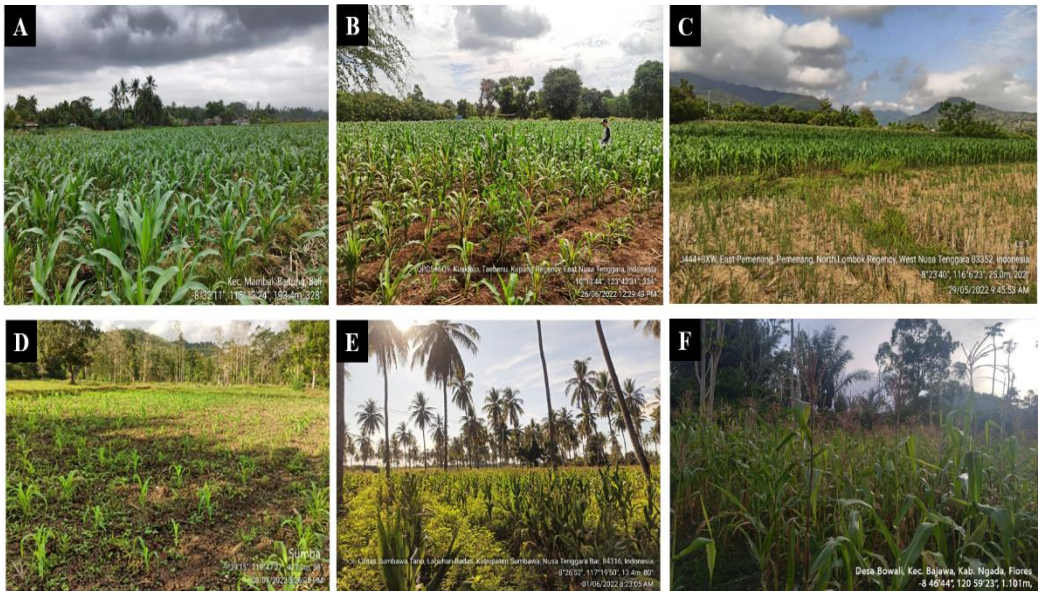

the coconut tree canopy, and between corn plantings it is also filled with peanuts. However, on the islands of Kupang, Sumba and Flores, corn is grown on dry land and is planted in monoculture (Figure 6).

Figure 6. Land conditions Corn plantations in each sampling location (Bali and Lesser Sunda provinces). Note: Bali (A), Kupang (B), Lombok (C), Sumba (D), Sumbawa (E) and Flores (F).

4. Discussion

The main factors affecting the distribution of insect species are biotic and abiotic elements [39–43]. Biotic elements which include the availability of host plants and the presence of natural competitors have a major influence on the high or low distribution of insect pests in agricultural land [44–46]. Abiotic elements in the form of differences in temperature, altitude, humidity, rainfall, and wind speed have a greater impact on the physiological processes of species, their distribution area, and their relationship with natural enemies [47]. It was reported that the potential for invasion of FAW pests in this study was very high in all sampling locations both in the Provinces of Bali and the Sunda Leser which still took into account the main host availability factor. Similar studies also reported that the presence of FAW is strongly influenced by environmental variables in various regions of China which include West Gansu, eastern Qinghai, Shaanxi, most of the Ningxia region, and parts of Tibet and more than 60% of the ideal distribution for FAW in this study is the western part of China whose distribution was simulated using ArcGIS and MaxEnt software [48,49]. The even distribution of FAW reported in this study is due to the availability of host plants that can be utilized by FAW to continue reproducing. Corn is one of the most widely grown agricultural commodities by farmers in the sampling area in this study.

The attack symptoms shown by FAW larvae in this study had similar characteristics to the symptoms found by several previous ones [3,13,50,51]. Attacks can be initiated by the larvae punching holes in the young leaves of the plant and then punching holes in the young leaves that are still curled up to cut off the growing point of the corn plant. Based on the search results, the distribution of FAW does not depend on the altitude of the location and these things, that FAW is able to adapt well to the altitude or geography and landscape found in the Sembalun Lombok isolate. This result is certainly different from previous findings that in the highlands > 5 masl there have been no symptoms of attacks from FAW and on the contrary high attack rates occur in the lowlands in Bali Province [13]. Insect invasion power is strongly influenced by the size and quality of plants produced from agricultural activities. Physical barriers (mountains, rivers and related environmental factors) and human agricultural activities have been able to influence the structure and distribution of insect populations to varying degrees [52,53].

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The results of the sequence analysis showed that the 9 isolates of FAW obtained from this study contained 2 isolates that had a similarity level of <50%, namely isolates from East Lombok and South Lombok compared to 7 other isolates. A total of three isolates (KB FAW, KP FAW, and FL FAW) had similarities with isolates from several regions in Indonesia including South Sumatra, Padang Pariaman, Tanah Datar and several isolates from several countries such as India, Bhutan, Vietnam and Pakistan with some strains such as Rice, Jadung and Sorghum. Meanwhile, four isolates (SB_FAW_Sorgum, SB_FAW_Maize, and JB_FAW_Maize) belonging to cluster I were close to 2 isolates (LT_FAW_Maize and LS_FAW_Maize) from cluster III. Interestingly, the two isolates from cluster III had different dominant nucleotide sequences compared to other isolates based on the results of the alignment analysis. The dominant difference in the nucleotide sequence of the two isolates can be attributed to mutations. This mutation has the potential to cause widespread insecticide resistance in consistently exposed target insects [54].

An epigenetic mechanism may be one of the causes of the appearance of differences in the nucleotide sequence arrangement in the two isolates which can cause genetic mutations. The influence of nutrition plays an important role in the epigenetic mechanism in insects [55]. Organisms including insects will certainly try to cope with the constant fluctuations of their biotic and abiotic environment. When these changes are felt and integrated, the organism has the potential to adapt its phenotype by modifying its physiology to then be able to increase its ability to deal with new environmental conditions and avoid stress [56].

Overall, the results of this study are able to provide preliminary information regarding the spatial distribution and the effect of location/geographical differences and host plants on the genetic diversity of FAW in the Provinces of Bali and Sunda Leser. However, it is still necessary to conduct further research on the effect of different host plants on the incidence and severity of attacks caused by FAW. Therefore, further research on the effect of different host plants on the incidence and damage by FAW still needs to be done. In addition, potential natural enemies and other biological control models also need to be studied comprehensively in controlling FAW attacks.

5. Conclusions

This study has found and confirmed data on the spatial distribution of FAW invasive insects in the Provinces of Bali and Lesser Sunda which are evenly distributed in each area covering low, medium to highland areas. Expansion and types of host plants such as corn, sorghum and rice planted by farmers in each region have the potential to be attacked by FAW. This study is the first to confirm the emergence of FAW through DNA Barcoding approach to 9 isolates obtained from the Provinces of Bali and Lesser Sundas. Phylogenetic analysis showed that three clusters with the highest nucleotide sequence differences were obtained by 2 isolates from East Lombok and Lombok. South with corn host plant strains that have similarities with isolates JB_FAW (Maize_Indonesia), SW_FAW (Maize_Indonesia), and SB_FAW (Maize_Indonesia). The FAW isolates obtained from this study have similarities with FAW isolates originating from several regions in Indonesia including South Sumatra, Padang Pariaman, Tanah Datar, West Pasaman and outside Indonesia including India, Bhutan, Vietnam, and Pakistan. Further comprehensive studies still need to be carried out, especially prudent efforts to control FAW which can be through the use of natural enemies, parasitoids, insecticides with active plant ingredients in order to support Integrated Pest Management (IPM) program in Indonesia.

6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

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