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Floribunda

Floribunda merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

Petunjuk kepada pengarang

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Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia. Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

Rujukan pembakuan

Pemakaian Bahasa Indonesia sepenuhnya mengikuti *Pedoman Umum Ejaan yang Disempurnakan, Pedoman Umum Pembentukan Istilah, Kamus Besar Bahasa Indonesia,* serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada *Oxford Dictionary of the English Language.* Ketentuan-ketentuan yang dimuat dalam *Pegangan Gaya Penulisan, Penyuntingan,* dan *Penerbitan Karya Ilmiah Indonesia,* serta *Scientific Style and Format: CBE Manuals for Author, Editor, and Publishers,* dan buku-buku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada *International Code of Botanical Nomenclature* bersifat mutlak.

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Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah tubuh teks tetapi sebelum daftar pustaka.

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

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GENETIC DIVERSITY OF LIMA BEAN (PHASEOLUS LUNATUS L.) FROM TIMOR ISLAND BASED ON MOLECULAR MARKER INTER-SIMPLE SEQUENCE REPEATS (ISSR)

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Emilia Juliyanti Bria & Polikarpia Wilhelmina Bani. 2021. Keanekaragaman Genetik Kacang Kratok (*Phaseolus lunatus* L.) Asal Pulau Timor Berdasarkan Penanda Molekuler Inter Simple Sequence Repeat (ISSR). *Floribunda* 6(7): 257–263. — Kacang kratok (*Phaseolus lunatus* L.) merupakan salah satu sayuran polong penting di Indonesia. Namun, informasi genetik tumbuhan ini masih sangat terbatas khususnya di pulau Timor. Penelitian ini bertujuan untuk menganalisis keanekaragaman genetik lima bean dari pulau Timor berdasarkan penanda molekular ISSR. Sebanyak 4 aksesi lima bean dianalisis menggunakan 3 primer ISSR menghasilkan 15 pita polimorfik dengan rata-rata 68,18 % polimorfisme. Hasil analisis klaster menggunakan metode Unweighted Pair Group Methods using Arithmetic averages (UPGMA) untuk mendapatkan dendogram yang menghasilkan dua klaster utama yakni kelompok biji warna polos dan kelompok biji warna berpola dengan koefisien similaritas 0.52. Hasil ini menunjukkan tingginya variasi genetik kacang kratok asal pulau Timor. Hasil ini juga memberikan metode yang bagus untuk mengevaluasi keanekaragaman genetik kacang kratok menggunakan penanda ISSR dan menyediakan informasi penting dalam budidaya kacang kratok di masa mendatang.
Kata kunci: Keanekaragaman genetik, *Phaseolus lunatus*, Timor, ISSR .

Emilia Juliyanti Bria & Polikarpia Wilhelmina Bani. 2021. Genetic Diversity of Lima Bean (*Phaseolus lunatus* L.) from Timor Island Based on Molecular Marker Inter-Simple Sequence Repeats (ISSR). *Floribunda* 6(7): 257–263. — Lima bean (*Phaseolus lunatus* L.) is one of the important legume vegetables in Indonesia. However, genetic information for these plants is still minimalized, especially on Timor Island. This study aims to analyze the genetic diversity of lima beans from Timor Island based on ISSR molecular markers. A total of 4 accessions of lima beans were analyzed using 3 ISSR primers to produce 15 polymorphic bands with an average of 68.18% polymorphism. The cluster analysis results use the Unweighted Pair Group Methods using Arithmetic averages (UPGMA) method to create a dendrogram that produces two main clusters. There were plain seed and pattern seed group with a similarity coefficient of 0.52. These results indicated that the genetic variation of the lima beans from Timor Island was high. Moreover, the result provides a sutable method for evaluating the genetic diversity of lima beans using the ISSR marker and important information of future lima bean breeding programs.
Keywords: Genetic diversity, *Phaseolus lunatus*, Timor, ISSR.

Lima bean (*Phaseolus lunatus* L.) is one of legume vegetable which contain essential acid (Kyeremateng 2015) and also has a high carbohydrate content with a low glycemic index (Rif'atunidaudina *et al*. 2019). This species is cultivated for seed purposes, and its usually used the young pods while dry seeds are rarely used because of the cyanide acid presence (Purwanti & Fauzi 2019; Rif'atunidaudina *et al*. 2019; Suryastini *et al*. 2019). This species originated from Central America and the Andes Mountains and then spread to Indonesia (mainly in Java) and commonly known as 'kratok' (Suryastini *et al*. 2019; Baudoin 1989, 2006). There were four groups of lima beans reported in Asia, namely 1) Java bean, its medium, purplish-red to black seed and contains large amount of HCN; 2) Red Rangoon/Burma bean, its small red seed with white spot and not contain

HCN; 3) white Rangoon/ Burma bean, its small white seed and not contain HCN; and 4) /Lima bean/kratok, its large white flat seed and not contain HCN (Baudoin 1989). In East Java, Purwanti (2014) recognized five beans and classified its into two main groups based on classification according to Baudet (1977), namely 1) medium-large size (sieva-big lima cultigroup) and 2) small-medium sized seed (potato-sieve cultigroup). The important characters used for identifying the diversity of Lima bean from East Java were weight, length, width, and thickness of seed, length, and width of leaves, and pod (Purwanti & Fauzi 2019).

Lima beans on Timor island are used by local people only as complementary legumes whose existence is rarely found. These beans are usually cultivated by rural communities and consumed at home only. Local people recognize two types of these beans, namely the poisonous type that grows wild and the cultivated type that can be consumed. The poisonous type can be consumed if it has been boiled repeatedly and usually during the lean season. Previous research conducted by Bria *et al*. (2019) revealed that there were two main clusters: (1) pattern seed and (2) plain seed five bean groups based on the morphological characters of 23 accessions collected including poisonous type toxic and cultivated types taken from various locations on Timor island.

Genetic diversity information is needed to support conservation and breeding activities of this plant. Alansi *et al*. (2016) revealed that assessment of genetic diversity is very important for the conservation of plant genetic resources in their natural habitat. Moreover, the potential germplasm breeding and conservation programs really need data on intra and interspecific genetic diversity of a species (Ben-Ying *et al*. 2010). Molecular markers are an effective technique in plant breeding programs and conservation of genetic resources. Inter Simple Sequence Repeats (ISSR) is a molecular marker that uses DNA-based PCR (Polymerase Chain Reaction) techniques with repeating sequence motifs and able to detect more specific DNA bands (Agisimanto *et al*. 2007). These molecular markers are generally used for the study of phylogeny diversity, gene tagging, genome mapping and evolutionary biology in various plants (Reddy *et al*. 2002). Several studies have used ISSR molecular markers in lima bean such as Martinez *et al*. (2008) worked on the genetic erosion and In situ conservation of Lima bean landraces in its Mesoamerican diversity center, Camacho *et al*. (2017) who reported on the genetic structure of Lima bean landraces grown in the Mayan area, and Nasir *et al*. (2021) who revealed genetic diversity analysis of Lima bean Landrace from Ethiopia.

The aims of this study was to investigate and describes genetic variability based on ISSR markers for the purposes of identifying and classification of lima beans from Timor island.

MATERIALS AND METHODS

Materials

Materials used for this study was lima bean accessions collected from the Timor island aged 3-4 weeks old (Table 1). Four plant accessions were taken from 23 plant accessions as a representative sample of the cluster formed from previous research (Bria et al., 2019). The sample determination used in this study was based on the results of genetic variability compared to the cluster morphologically.

The leaf samples used in molecular analysis were taken from the ± fifth leaf from the plant top. Other materials used for molecular study were 70% alcohol, DNA extraction kit (Nucleon Phytopure), cold chloroform, cold isopropanol, 70% cold ethanol, sterile aquabides, TE buffer, aquades, 10X TBE buffer (0.89 M Tris Borec Acid, 0.02 M EDTA Disodium, pH 8.4), agarose powder, DNA ladder (Vivantis 100 bp), loading dye, DNA Staining flourosafe (Sybr Safe DNA Gel Stain Invitrogen), DNA PCR Kit (2x My Taq HS Red Mix Bioline), ice 23 gell, and the ISSR Primer (Table 2).

Table 1. Plant materials

No.	Accession	Morphological characters
1	A2	The main stem and each node is pigmented, purple flower, plain black seeds, medium size, oval-round shape
2	A4	Non-pigmented stem, white flower, plain white seeds, medium size, oval-round shape
3	B2	Non-pigmented stem, white flower, seed white with spot pattern of maroon color, big size, kidney shape
4	B4	Non-pigmented stem, white flower, seed white with spot pattern of maroon, small-medium size, oval-round shape

Molecular analysis was performed in three stages, i.e., DNA isolation, DNA amplification via polymerase chain reaction (PCR) technique, and amplification visualization.

DNA Isolation

Procedure of DNA isolation was carried out using the Nucleon Phytopure Kit and following the Nucleon Phytopure Kit protocol based on the procedure describe by Bani *et al*. 2017 with modifications in reagent volume, clorophome number dan resin number. Firstly, the lima bean leafs weighed as much as 0.3 grams, then crushed with a mortar and grinder until smooth and 600 µl of Phytopure I reagent was added. The results of the grinding were mixed and put in a 1.5 ml tube. The mixture was then given 200 µl of Phytopure II reagent and inverted several times until it was homogeneous. The mixture was then incubated at 65^{o}C for 10 minutes, then transferred to an ice box for 20 minutes. After that, 500 µl of cold chlorophome and 100 µl of Phytopure Resin were added to the mixture, then shaken for about 30 minutes. The mixture was then centrifuged at 10,000 rpm for 10 minutes, to obtain the supernatant and pellets. The supernatant transferred into a new 1.5 ml tube and added isopropanol then centrifuged at a minimum speed of 10,000 rpm for 10 minutes. The pellets obtained were then washed with 100 µl of 70% ethanol and centrifuged again at 10,000 rpm for 5 minutes. The isolated DNA was then resuspended with the addition of 50 µl TE 1X and stored in cold conditions (-20^{o}C).

Table 2. ISSR primers used in this study

Primer	Sequence (5' – 3')	Number of bases	Annealing temperature
ISSR8	AGA TAG ATA GAT AGA TAG ATG Y	22	49,2^{o}C
UBC-841Y	GAG AGA GAG AGA GAG AYC	18	44,9^{o}C
H-814	CTC TCT CTC TCT CTC TTG	18	43^{o}C

Amplification-PCR

PCR was done by first making a PCR mix (Table 3) according to the My Taq Redmix (Bioline) PCR kit protocol. The amplification begins with predenaturation at a temperature of 94^{o}C for 4 minutes, then followed by 40 cycles consisting of a denaturation step of 94^{o}C, for 1 minute, annealing 44.5^{o}C–51.5^{o}C (depending on the type of primer) for 1 minute, elongation at 72^{o}C for 90 seconds and post elongation at 72^{o}C for 10 minutes.

Visualization

The amplification products were separated by electrophoresis on 1.8% agarose gel at a voltage of 50 volts for 50 minutes. DNA band visualization was performed using a UV light transilluminator. PCR-ISSR data analysis used the visualization of electrophoretic DNA bands and then photographed using optilab.

Table 3. Mix PCR composition

Material	Volume for *Mix PCR* (µl)	Final concentration
ddH2O steril	6	-
Bioline 2x My Taq HS hotstart	12,5	1x
Primer	2	10 ng/ µl
$MgCl_2$	2,5	
DNA template	2	200 ng/ µl
Total	25 µl	

Data Analysis

Determination of genetic diversity, the results of PCR-ISSR of molecular characters qualitatively using genomic bands were carried out by comparing the size of the DNA bands formed with the genome size of the DNA ladder. The DNA band size was determined by measuring the distance of each band formed using the Image Raster 3 computer program which was compared with the distance and size of the DNA ladder and then ana-

lyzed using Microsoft Excel (.xls).

ISSR data were analyzed based on the presence (1) or absence (0) of the visible DNA bands. Jaccard coefficients was used to calculate similarity index. The cluster method algorithm Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) was used to construct dendrogram using the Multivariate Statistical Program (MVSP) software version 3.1.

RESULT AND DISCUSSION

The results of the ISSR analysis in this study using 3 primers, 22 amplified bands showed that the four accessions of lima bean from the Timor Island had band sizes ranging from 250 bp–900 bp (Figure 1) with 15 polymorphic characters and 6 monomorphic characters (Table 4). The primer that produced the most bands was ISSR8 which produced 8 bands, while the other two primers (UBC-841Y and H-814) produced 7 bands each. Of the 22 bands produced, 15 bands were polymorphic (68, 18%) and all these primers produced polymorphic bands greater than 50% ranging from 57.14% –75%. This result is supported by Nasir *et al*. (2021) who reported that out of 106 loci generated by eight primers 95 (88.2%) of them were polymorphic on Genetic diversity analysis of Lima bean landrace from Ethiopia. This is consistent with Martinez *et al*. (2017) who reported on the genetic structure of Lima bean landraces grown in the Mayan area using four ISSR generated 75 bands, where all them (100%) were polymorphic. In addition, Martinez *et al*. (2008) worked on the genetic erosion and In situ conservation of Lima bean landraces in its Mesoamerican diversity center and out of the 90 bands analyzed 71 (78.8%) were polymorphic.

Table 4. Result of percentage polymorphism *P. lunatus* L. from Timor Island

Primer	Total character	Polymorphic	Monomorphic	% Polymorphism
ISSR8	8	6	2	75
UBC-841Y	7	4	3	57,14
H-814	7	5	2	71,43
Total	22	15	6	203,57

The largest and the smallest band sizes were all seen in the ISSR8 primer, which was 900 bp in accessions A2 and A4 while the smallest size was 250 bp in accessions B2. In the ISSR8 primer, the band sizes that appeared in all accessions were 400 bp and 500 bp. In the UBC-841Y primer, the band sizes that appeared in all were 390 bp, 500 bp, and 550 bp, while in the H-814 primer the band sizes that appeared in all accessions were 400 bp and 500 bp. Djuita *et al*. (2020) revealed that bands of a certain size that frequently appear in a primer suggest that these bands may be bands common to the species. In this study, it can be seen that the band that can be found in all accessions and primers is 500 bp. The band size which was only owned by one accession was 250 bp in the ISSR8 primary B2 accession and 300 bp in the H-814 primary A4 accession. This can be one of the important characters in the identification process. The primers used in this study produced quite diverse bands between accessions and between primers. This shows that the primers used are able to amplify the observed plant genome sequences (Rif'atunidaudina *et al*. 2019).

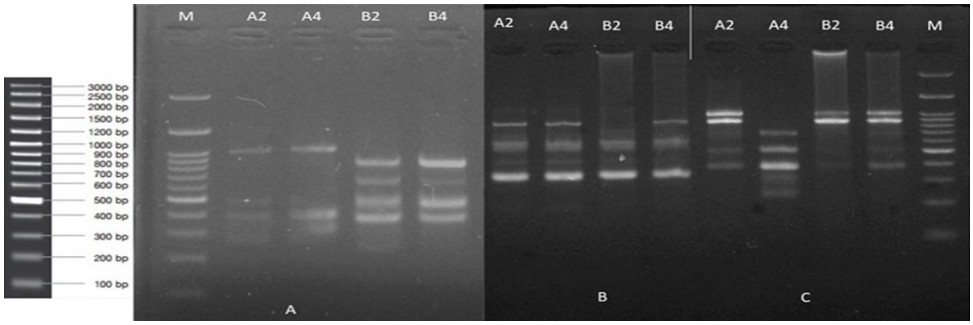

Figure 1. ISSR Patterns of *P. lunatus* L. from Timor Island. Primer ISSR8 (A), primer UBC-841Y (B), primer H-814 (C)

Variations in polymorphic bands can be caused by several things, such as differences in plant types, taxon levels, the number of samples analyzed and the number of ISSR primers used. The more varied the populations, the number of samples and primers used for analysis is thought to result in a higher level of polymorphism (Hariri *et al.* 2017). In this study, the most common polymorphic bands appeared in ISSR8 primers but did not differ much from the others (Table 4). This discussion shows the importance of knowing the profile of bands of a certain primer in study of the genetic variability of a given species (Rocha *et al*. 2014).

Based on molecular characterization, dendrogram shows that lima beans from Timor island consist of two main clusters, A and B, at a Jaccard's similarity coefficients of 0.52 (Figure 2). Cluster A consisted of accessions with a similarity coefficient of 0.66, which had a pattern of seed color but different sizes and shapes of seeds. Cluster B consisted of accessions that did not have a seed color pattern (plain color), but the size and shape of the seeds were the same at similarity coefficient 0.72. The seed size did not really have an effect on clustering, but it is important given in between A2 and A4 accessions is closer than B2 and B4. The clustering pattern in this study was closely related to the bisexual papilionaceous five bean flower structure with unopened buds, favoring self-pollination of about 52% and causes gene flow among populations is low (Nasir *et al*., 2021).

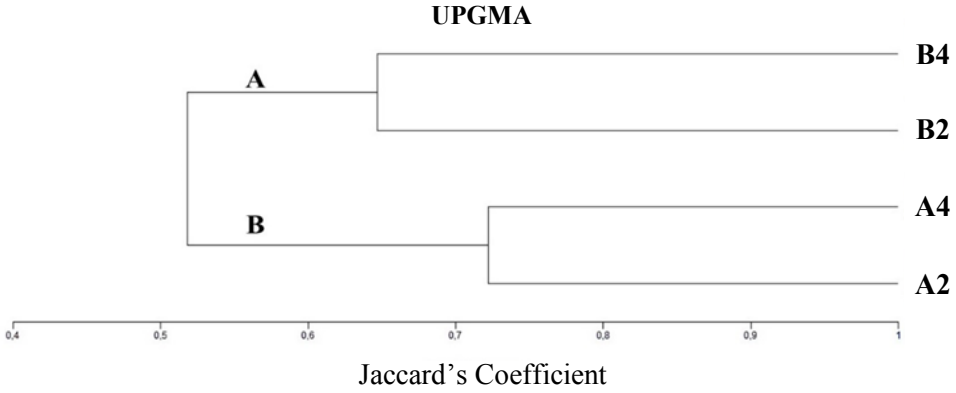

Figure 2. Dendogram of relationship among 4 accession of lima bean according ISSR marker .

Camacho-Pe´rez *et al*. (2017) stated that the structure and genetic relationship of the Mesoamerican gene pool using 73 ISSR loci could also be distinguished based on altitude, but this difference is not significant. In addition, Nasir *et al*. (2021) reported a very strong grouping among individuals collected from the same zones and geographically distinct zones using Jaccard's similarity coefficients were used to construct the UPGMA dendrogram for 96 accessions of lima bean landrace from Ethiopia based on the bands obtained with the eight primers ISSR markers. However, the effect of location does not apply in this study. The separation and formation of a cluster between accessions may suggest that accessions may have been isolated from each other for a longer period in time and as a result there was limited gene flow due to long distance. This is also influenced by the existence of lima beans on the island of Timor which are only cultivated by a small number of farmers for home consumption.

The results of this study support previous research (Bria *et al*. 2019), which classifies lima bean from Timor Island into two main clusters based on morphological characters. Although Timor Island is not the center of the diversity of lima bean, the results of this study indicate that lima bean from Timor Island has high genetic diversity. This is the basis for the development and conservation of this germplasm and help in future plans for conservation and sustainable use of lima bean genetic resources of the country.

CONCLUSION

Based on molecular characters lima bean from Timor island was divided in two main group. They were 'plain' seed group and 'pattern seed group. Furthermore, the obtained results indicated that the lima bean genotypes investigated in this study have wide genetic diversity. For better understanding of genetic diversity of lima bean, future studies

should focus on a larger number of populations and accessions collected from more geographical region because different methods of sampling genetic variation allow sampling at different levels and differ in their power of genetic resolution as well as the quality of information content.

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