

Genetic diversity and proximate analysis of Indonesian local mung bean (*Vigna radiata*)

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Abstract. Afza H, Palupi ER, Herlina L, Ilyas S. 2023. Genetic diversity and proximate analysis of Indonesian local mung bean (*Vigna radiata*). *Biodiversitas* 24: 6377-6388. Mung bean is an important food crop because it has a high protein content. However, not much information is available about Indonesian local mung beans. This study aimed to obtain information about flowering and seed formation in local mung beans (*Vigna radiata* L.) to determine the genetic diversity of mung beans using simple sequence repeats and the protein, starch, fat, and ash content. Mung bean seeds of 15 genotypes were obtained from the Indonesian Agricultural Gene Bank collection. The experiment was arranged using a one-factor randomized complete block design. Genotypes with a low degree of non-synchrony of pod maturity (DDd) were Local Bima and Arta Koneng 01070. The highest fruit set was observed in the Madura Local accession (73.09%). The accumulated value of heat units was calculated as growing degree days; the heat units from planting to the first flowering ranged from 544.6-670°C and from planting to the first ripe pod (heatd2) and 90% ripe pod (heatd3) phases ranged from 819.7-933.7°C and 1,111.9-1,419.1°C. Phylogenetic tree construction was made based on scoring of the presence of alleles that appeared on the electrophoresis results of 12 microsatellite markers on 15 mung bean genotypes. The superior varieties, Vimal and Walet, were separated from the other 13 local genotypes at a genetic similarity coefficient of 0.583. The protein contents of the tested mung bean genotypes were 19.19%-23.06%.

Keywords: Fruit set, heat units, indeterminate, microsatellites, SSR

INTRODUCTION

Mung bean (*Vigna radiata* L.) is an important food crop because it contains protein, carbohydrates, and various micronutrients (Guleria and Kumar 2017; Yi-Shen et al. 2018; Sahoo et al. 2020; Shrestha et al. 2023). Mung beans have a high content of essential amino acids, including branched-chain amino acids (Ebert et al. 2017; Nasrollahzadeh et al. 2023; Yanti et al. 2023). However, the consumption and production of mung bean are lower than that of soybean. Indonesia's soybean harvest area was 680,373 hectares, mung bean harvest area was 197,508 hectares, and production was 234,720 tons. This figure does not cover the annual demand for mung beans, which is 304,000 tons (Ministry of Agriculture 2021). The soybean harvest area in Indonesia in 2021 was 680,373 ha, while the mung bean harvested area was 197,508 ha, with a production of 234,720 tons.

One of the reasons for farmers' lack of interest in mung beans is their indeterminate and semi-determinate growth, which causes flowering to occur at different times (non-simultaneously). After the initial pods are ripe in indeterminate and semi-determinate development, they are followed by the next flowering (Talukdar et al. 2020; Van Haeften et al. 2023). Therefore, the pods do not ripen concurrently in one crop cycle (Ha et al. 2020). This asynchronous pod ripening

causes harvesting in stages based on pod maturity, which raises harvest costs (Ahsan et al. 2014; Iqbal et al. 2015).

The lack of interest among farmers in growing mung bean, coupled with the use of new superior varieties, has made local genotypes of mung bean a group of plants that are increasingly rarely cultivated by farmers, putting them at risk of genetic erosion or extinction, even though local genotypes are valuable genetic resource in assembly programs. Local genotypes are important in expanding the genetic diversity of cultivated plants (Kim et al. 2015; Nikolova and Georgieva 2017; Pataczek et al. 2018; Kalapchieva et al. 2020).

Information on flowering phenology and fruit development is required in the development and use of plants, for example, in increasing seed production and quality through the right time and method of harvest, determining the application time of herbicides and pesticides, as well as in assembling superior varieties (Al-Khayri et al. 2019). Until now, not much research has been conducted on the flowering patterns and reproductive capacity of diverse mung bean varieties (Somta et al. 2022), particularly local varieties or unreleased accessions (González-Suárez et al. 2020; Lee et al. 2021). Therefore, comprehensive phenological studies and characterization of various germplasm will be beneficial in exploring biodiversity

to generate new superior varieties (Gayacharan et al. 2020; Tabasum et al. 2020).

In phenology studies, a quantitative method links plant growth and development with daily average temperatures compared to plant base temperatures (Elnesr and Alazba 2016; Khan et al. 2020). The base temperature of the mungbean plant is 10°C (Singh and Singh 2015). The base temperature varies among crops, and the value is derived from the growth habits of each specific crop. The base temperature of mungbean (10°C) means that the temperature below 10°C plant growth is zero, and no significant development of mungbean is expected. The accumulated value of heat units is calculated in terms of growing degree-days (GDD) (Akyuz et al. 2017). This quantitative method is used in several phenological studies and predicted planting and harvesting dates, plant stadia length, and maturity level (Elnesr and Alazba 2016).

In managing genetic resources and plant breeding, the study of genetic diversity is very important, such as ascertaining and tracing the background of these plants (Tripathy and Das 2021; Singh et al. 2022; Jiang et al. 2023). One way to determine plant genetic diversity is by using molecular analysis based on the polymorphism of a species based on the differences in the resulting DNA bands. Analysis of genetic diversity with molecular markers was not influenced by environmental factors and plant stadia (Thakur et al. 2017; Mamo et al. 2023). Minisatellites have 10 to 100 tandem repeats, and microsatellites have less than ten base pairs. Both minisatellites and microsatellites have been identified in the sequenced mitochondrial genomes of several plant species and have formed the basis of PCR-based polymorphic markers. (Grosser et al. 2023). The category of tandem repeats in which the repeat unit is longer than 100 bp is defined as the satellite (Chen et al. 2023). Simple sequence repeat (SSR) is a microsatellite with several advantages compared to other molecular

markers. These microsatellites allow the identification of many alleles at a single locus and are evenly distributed throughout the genome (Vieira et al. 2016; Zavinon et al. 2020; Corvalán et al. 2023; Topu et al. 2023).

This study aimed to determine plant growth patterns, production potential, proximate analysis of mung bean seeds, and genetic diversity based on the SSR of several mung bean genotypes in Indonesia. The information obtained will be useful in mung bean breeding and seed production.

MATERIALS AND METHODS

Time, research location, and plant material

The research was conducted from January 2022 to October 2022. Therefore, 15 mung bean genotypes consisted of 2 superior varieties (Vimal and Walet) as a comparison, 1 introduced genotype from AVRDC Taiwan (Pag Asa 2), and 12 Indonesian local genotypes. The seeds come from the Indonesian Agricultural Gene Bank collection, planting location at the Bogor Cikemeuh BB Biogen Experimental Station, Bogor City, West Java. Geographically, the Experimental Station is located at 6.574998 South Latitude, 106.787329 East Longitude, with an altitude of ± 200 m above sea level. The daily climate data used in this research comes from the BMKG West Java Climatology Station, Bogor City.

Experimental design

The experiment was arranged based on a one-factor randomized complete block design and 3 replications, so the number of experimental units was 45. Each experimental unit consisted of five sample plants, with 225 observation units. Seeds of each mung bean genotype were planted with a spacing of 40 cm x 40 cm.

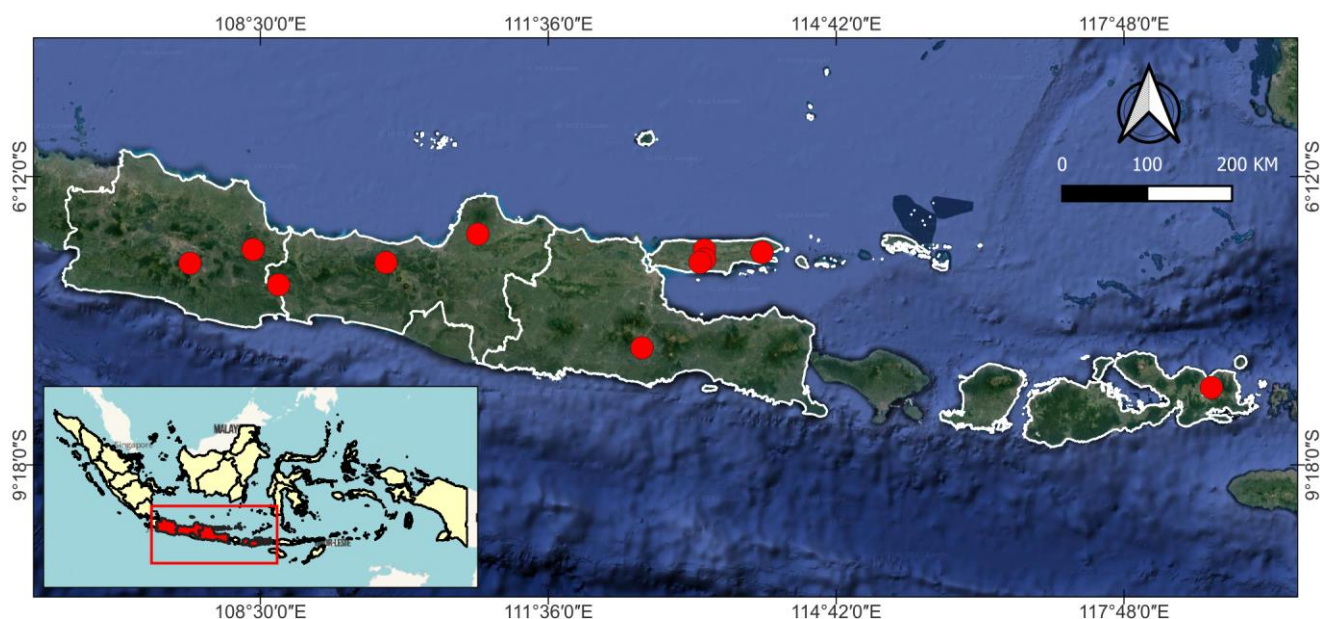


Figure 1. Map of the area of origin of Indonesian local mung bean seed collections

Table 1. Degree of unsynchronized pod ripening and indeterminacy of plant height

Description	Code	Information/formula
The first day of flowering	D1	
The first day of maturity of the first pod	D2	Marked by a change in the color of the pods from green to brown or black
Pod maturity 90%	D3	
Plant height (cm) at the first flower	H1	
Plant height (cm) when the first pod ripened	H2	
Plant height (cm) at pod maturity 90%	H3	
Degree of non-synchrony of pod maturity (DDd)	DDd	DDd from the first flower to pod maturity 90% (DDd1) $DDd1 = \frac{D3-D1}{D3} \times 100$ DDd from the first pod maturity to 90% pod maturity (DDd2) $DDd2 = \frac{D3-D2}{D3} \times 100$
Degree of indetermination for plant height (DDh)	DDh	DDh from the first flower to the first pod maturity $(DDh1) = \frac{H2-H1}{H2} \times 100$ DDh from the first flower to ripe pod 90% $(DDh2) = \frac{H3-H1}{H3} \times 100$ DDh from first pod cooking to 90% pod maturity $(DDh3) = \frac{H3-H2}{H3} \times 100$

Diversity of plant growth patterns, production potential, and chemical composition

Flowering time, first pod formation, and 90% ripe pods

Observations began during flowering, pod formation, and pod ripening, based on days after planting (DAP). Flowering pattern, degree of pod maturity asymmetry, and degree of indeterminate were observed with the criteria according to Khattak et al. (2002, 2004) (Table 1).

Evaluation of plant development based on the heat units needed to reach the flowering phase, pod formation phase, and ripe pods by calculating the number of heat units (heat units = HU) received by the plant. The number of heat units is calculated based on growing degree days (GDD). In this study, GDD was calculated using the basic temperature (Tbase) of mungbean plants of 10°C (Singh 2015), with the appropriate equation (Singh and Singh 2015; Elnesr and Alazba 2016; Khan et al. 2020) as follows:

$$HU = \sum_{i=1}^n GDD_i$$

$$GDD = \text{MAX}\{0.5(T_x + T_n) - T_b\}$$

Where:

T_x : Daily maximum temperature (°C) for a day

T_n : Daily minimum temperature (°C)

T_b : Plant base temperature

Mung bean seeds require temperatures of at least 15°C at the start of planting, and optimal temperatures are 20–30°C, with an average temperature of 28°C during crop production (Khan et al. 2020). The base temperature is 10°C and the maximum temperature of 40°C. The temperature outside the base and the maximum temperature will cause mung beans to stop growing/plants cannot grow (Chauhan dan Williams 2018).

Production potential

Production potential was evaluated based on the percentage of pod formation (the proportion of pods that successfully matured from all the flowers formed), the weight of 100 seeds, and the weight of the seed harvest. In the generative phase of mung bean plants, the number of flowers in the first harvest, the number of pods formed in the first harvest, the number of flowers that appeared in the second harvest, and the number of pods formed in the second harvest were counted (Chaves et al. 2020; Atasagun et al. 2021).

Fruitset = (pods/panicles) : (flowers/panicles) or pods/total flowers

Proximate analysis

Proximate analysis was carried out to determine the content of important seed compounds, namely protein, fat, starch, ash, and moisture content. Proximate analysis was conducted at the Starch Laboratory, National Research and Innovation Agency (BRIN), according to The Indonesian National Standard SNI 01-2891-1992. The samples were fresh local mung bean seeds harvested from plantations at the Cikemeuh Experimental Station, Bogor.

Genetic diversity based on molecular markers in mung bean plants

Marker analysis

Molecular characterization with SSR markers was carried out at the ICABIOGRAD Molecular Biology Laboratory. The materials used in DNA isolation included leaf samples from each genotype of mungbean plants (15 samples in total), extraction buffer (cetyl trimethyl ammonium bromide (CTAB), 2%, 100 mM Tris-HCL pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, polyvinylpyrrolidone (PVP) 2%, sodium disulfite 0.3%), mercaptoethanol, chloroform-

isoamyl alcohol solution (24: 1), Na-acetate, isopropanol, 70% ethanol, double distilled H₂O (ddH₂O). The materials used for electrophoresis include acrylamide (acrylamide, acrylamide bis), APS (ammonium persulfate), TEMED (tetramethylethylenediamine), tris borate EDTA (TBE) buffer, gloves, 1.5 mL tube, tube 2.5 mL, pipette tips, label paper, distilled water, ethanol, RNA-ase, isopropanol, 70% ethanol.

Materials used for qualitative test: loading dye, ladder (100bp size), SYBRgold, 8% polyacrylamide gel, 70 alcohol %, gloves, mask, 1.5 mL tube, 2.5 mL tube, micropipette, aluminum foil, tissue Kim wipes, APS (ammonium persulfate) solution, TEMED (Tetramethyl ethylenediamine) solution and parafilm. Materials used to amplify SSR markers: 12 primers (Table 2), buffer, dNTP, MgCl₂, TaqPolymerase, ddH₂O, and DNA samples. Each amplified sample has a total volume of 10 µL containing 10 ng/µL template DNA of 1.5 µL; 2x MyTaq HS as much as 5 µL; 1 µL of primer consisting of Forward and Reverse with a concentration of 5 µM, each of 0.5 µL; and 2.5 µL sterile ddH₂O.

Quantitative test for DNA carried out using a Nanodrop 2000 Spectrophotometric (ThermoScientific, USA). Calibration was carried out before taking measurements by measuring 1.5 µL of TE buffer blank. 1.5 µL of mung bean DNA was then measured for its concentration. The A260/280 ratio was used to determine protein contamination of a nucleic acid sample (Thermo Scientific 2016; Watts 2017). Nucleic acids absorb UV light at 260 nm due to the aromatic base moieties within their structure. The aromatic proteins have a strong UV absorbance at 280 nm. The measurement results will be visible on the computer with the ND-1000 Software.

Table 2. List of primers

Primer	Sequence (5'-3')
MBSSR136	F 5'ATGATGAGGTCGCAAGAGGG3' R 5'TCTACCGTGCAGTCGTCGC3'
MB SSR 021	F 5'ACATCCGGGAACAAACAAACG3' R 5'AACTGAGGCTTGAGAAGATGAC3'
DMB SSR 013	F 5'ACACAGATCATCATCACCAATC3' R 5'ACACAGATCATCATCACCAATC3'
VrD1	F 5'CAGCTTCTTGTCTTGCTCC3' R 5'AGTGAGAGAATGGTCAGTGG3'
MBSSR063	F 5'TGCTTGTAGGTCTGGTTATG3' R 5'CTGCACAACCAACAAACTC3'
MB SSR 179	F 5'ATTCAGGAGCACACTCTCC3' R 5'CTTAATATGATGGGTTGGCC3'
MBSSR 203	F 5'GTGCGTAATATGTGTGATGG3' R 5'CACATTCAACACGTACAAATAC3'
MBSSR 033	F 5'CTATTCTGAGTGCAGGTTTC3' R 5'GTGTGTGTGTTCTCGTGTGT3'
MBSSR 015	F 5'ATCATCATGACTCCGACACTC3' R 5'GTCGCGTAGCATGTTGGAG3'
GMES 1604	F 5'GTTGCAGGCACACTGGAGTA3' R 5'CTCAGCCTTCCTTCCCTGTTG3'
GMES 2225	F 5'CCTCCTAATGAGGCCAATGA3' R 5'ATTATTCGGCCAACTTCC3'
Gaat 47	F 5'TGTCCATGTTTAGTGATGAGGC3' R 5'CTGTTGTGATCGGAAGGTGTAG3'

The parameter for selecting the best DNA at an A260/A280 absorption purity ratio of approximately 1.8 is generally accepted as pure for the A260/A280 DNA absorption purity ratio. Good quality DNA will have an A260/A280 ratio of 1.7 to 2.0. The absorbance at 260 nm and the A260/280 purity ratio value were reproducible when using low-salt buffer as the elution buffer rather than water (Thermo Scientific 2016; Watts 2017).

The polymerase chain reaction (PCR) reaction used the PCR T1 Thermocycler machine (Biorad) with the following program: initial denaturation at 94°C for 3 minutes, then 35 cycles of the denaturation process at 94°C for 45 seconds, annealing (primer attachment stage) at 55°C for one minute, and extension at 72°C for one minute. The last stage/final extension cycle (final stage of base extension) was at 72°C for 10 minutes and incubation at 4°C for 3 minutes. Then, the sample was electrophoresed using 8% polyacrylamide gel in a tank containing TBE buffer.

From the results of DNA electrophoresis, the heterozygosity (H) of mung bean was determined using the following Hardy-Weinberg equation:

$$H = 1 - \sum_{i=1}^n p_i^2$$

Where:

n : number of alleles

p_i : frequency of occurrence of allele i

The Polymorphic Information Content (PIC) value was obtained using the following formula (Sunayana et al. 2017):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i^2 p_j^2$$

Where:

n : number of alleles

p_i : frequency of occurrence of the ith allele

p_j : frequency of occurrence of the jth allele

The software program POPGENE 1.31 (Yeh et al. 1999) was used to obtain the genetic diversity parameters: the number of effective alleles (Ne) value, the Ne'si diversity index (h), and the Shannon Index (I).

Cluster analysis of SSR

The visualization results on the polyacrylamide gel were scored as binary data. Each amplified band was considered one allele and was given a score of one, while bands that were not visible were given a score of zero. Bands with the same movement rate were considered the same locus, seen in the visualization results as one allele, resulting in binary data. Data were analyzed using the Sequential Agglomerative Hierarchical and Nested (SAHN)-UPGMA (Unweighted Pair-Group Method with Arithmetic) program on the NTYSYS version 2.1 device. The scoring results were processed into the program and analyzed through the Similarity-Qualitative data-Clustering-SAHN-Graphics-Tree plot menu to obtain phylogenetic dendrogram data and the genetic similarity matrix.

RESULTS AND DISCUSSION

Flowering time and degree of non-synchrony of pod maturity (DDd)

Based on the time of first flower appearance (D1 phase), mung bean plants can be categorized as early maturing if the first day of flowering ≤ 40 DAP, medium category: $40 < \text{flowering} < 50$ DAP, and age > 50 DAP (Patel 2019). Based on these criteria, the 12 genotypes studied were early maturing (35-38 DAP), including 2 control varieties and 1 introduced variety, while 3 genotypes, Local Gadis, Arta Koneng 01070 dan Local Sumenep, were categorized as medium (41-42 DAP) (Table 3).

Nair et al. (2012) stated that the early maturing category includes mung bean plants with a flowering time of ≤ 60 DAP. Based on this criterion, all the genotypes studied were early maturing (fast flowering) mung bean plants. The first flowering period of the local mungbean was generally longer than the new superior varieties (VUB: Vimal and Walet). However, the results of this study indicated that the first day of flowering for most of the local genotypes was not significantly different from VUB, except that three genotypes were slower than VUB. Presumably, because this local variety has experienced domestication and has been cultivated in its place of origin for a long time, it has undergone a natural selection process.

Based on the time of the first ripe pods (D2 phase), 6 genotypes were slower, ranging from 57-59 DAP (Mentik Coklat, Local Bima 2A, Local Gadis, Arta Koneng 01070, Local Sumenep dan Local Madura compared to other genotypes, ranging from 52- 55 DAP (Vimal, Walet, Local Batang, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Pag ASA 2, and Local Sampang) (Table 3). This data shows the duration of flower development from bud to filling of the pod. The genotypes that flower earlier are not always followed by earlier pod formation and pod maturity, which are influenced by genetic and environmental factors (Kozlov et al. 2020).

Based on 90% pod ripening time (D3 phase), 13 genotypes (Vimal, Walet, Local Batang, Mentik Coklat, Local Bima 2A, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Local Gadis, Arta Koneng 01070, Pag ASA 2, and Local Sampang) belong to the group with faster ripening pods than the other two genotypes (Local Sumenep and Local Madura) (Table 3). The genotype that produces pods ripening 90% faster is the preferred genotype group because it harvests faster. Faster harvest time will also impact reducing production costs in the field.

The results of data analysis on the degree of non-synchrony of pod ripening relative to first flower release and first pod ripening showed significant differences between mung bean genotypes. The desired mung bean genotype is the mung bean, which has a low degree of non-synchrony. The degree of non-synchrony of pod maturity from the first flowering to 90% of pod maturity (DDd1) indicates that the genotypes Local Bima 2A, Local Gadis, and Arta Koneng 01070 are more synchronized than the other genotypes (Table 3). These data indicate that flowering and pod ripening were more uniform in these three

genotypes, which were not significantly different from the Mentik Coklat, Local Sampang, and Local Sumenep genotypes.

Based on the degree of non-synchronous pod ripening from the first ripe pods to 90% ripe pods (DDh2), it showed that the genotypes of Mentik Coklat (DDd2=18.9%), Local Bima 2A (DDd2=20.6%) and Arta Koneng 01070 (DDd2=18.8%) were more simultaneous than the Local Taragong and Local Madura genotypes. The value of the degree of non-synchrony (DDd) from the first pod ripening to 90% pod maturity or $DDd2 \leq 20\%$ indicates the synchrony or the same harvest category (Tah and Saxena 2009; Marwiyah et al. 2021). These data indicate that the filling process for the 3 genotypes was more simultaneous than the other groups.

The synchrony of flowering and pod ripening is one of the important characteristics favored by mung bean cultivators because harvesting will be easier and save more time and effort. The measurement degree of the non-synchrony of the two methods is relatively consistent. The two measurements' correlation coefficient (DDd1 and DDd2) was obtained ($r = 0.7502$). It means that if the non-synchrony based on the first flowering is low, then the non-synchrony of the first ripe pod also tends to be low (Figure 2).

Plant height and degree of indetermination for plant height (DDh)

Data analysis using ANOVA and Tukey test for data on plant height at the first appearance of the flower and ripe pod and 90% ripe pod showed significant differences among the mung bean genotypes tested in this study. Genotypes that have short stems when the first flower appeared were Vimal, Walet, Local Batang, Mentik Coklat, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Pag ASA 2, Local Sampang, and Local Madura. At 90% pod maturity, the genotype groups were short based on the Tukey test, namely Vimal, Walet, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, and Local Sampang.

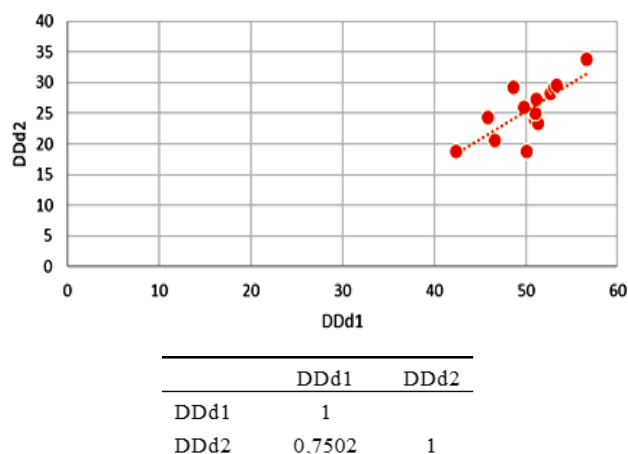


Figure 2. The correlation coefficient between DDd1 and DDd2

The categorization of plant height when the pods ripen according to Patel (2019), namely: short: <50 cm; medium: 50-70 cm; high: >70cm. Based on this category, the test genotypes are grouped into short plant group (Local Sampang), medium plant group (Vimal, Walet, Local Batang, Mentik Coklat, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Local Gadis, Pag ASA 2, and Local Madura). Genotypes Local Bima 2A, Arta Koneng 01070, and Local Sumenep are high because they have plant heights above 70 cm. The characteristics of mung bean plants that are superior in plant cultivation are fast harvesting and short plant stems.

From the results of the Tukey test for the degree of indetermination in plant height, there was no significant difference from the 15 genotypes tested. Although the degree of indetermination was not significantly different, there was an increase in plant height that varied between genotypes. In general, the degree of indetermination of plant height at the time of filling the pods (DDh3) has a lower value than the degree of indetermination of the first flower to the first pod maturity (DDh1) and the first flower to 90% pod maturity (DDh2). When filling the pods, the plants allocate most of the assimilate to form pods and seeds so that the rate of increase in plant height, leaf area, and other vegetative growth is quite low during pod filling. Research (Geetika et al. 2022b; Geetika et al. 2022a) states that more than 90% of assimilates or sinks will be allocated for pod formation during the pod-filling phase.

According to (Li et al. 2018), genotypes of high-yielding mungbean varieties generally have determinate properties, and all wild mungbean/landrace germplasm have indeterminate properties. The generality of superior and landraces or local varieties, but there are still variations in the degree of indetermination for the local genotypes.

According to the degree of indetermination, a category approach was used (Ullah et al. 2012; Iqbal et al. 2015; Marwiyah et al. 2021), which states that a plant is categorized as determinate if it has a DDh value of <38.5%. So if we refer to this category, the genotypes are Vimal, Local Bima 2A, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Local Gadis, Arta Koneng 01070, Pag Asa 2, Local Sampang, Local Sumenep including mung bean plants which are determinate.

The main criterion is the apical meristem to determine whether a plant is determinate or indeterminate. Suppose the apical meristem is terminated by flowering. In that case, it is classified as a determinate plant. However, if there is still growth on the main meristem after the flowering process and continues to the next flowering, it is classified as indeterminate (Rodas et al. 2023).

Heat units

The local mung bean genotype used in this study is a collection of the Indonesian Agricultural Gene Bank originating from exploration and collections from several regions in Indonesia. Not much has been done to describe and characterize local accessions, and the local government has yet to register varieties. Information on the number of heat units from local accessions is needed to determine planting standards if the local accessions are to be brought

for planting in other areas with different daily climates from their origin.

The results of heat analysis for the three phases, namely at first flowering, first ripe pod phase, and 90% ripe pod phase, showed significant differences between mung bean accessions. The heat units from planting to the first flower out (heatd1) ranged from 544.6-670.0°C. Mung bean cultivation from planting to the first ripe pod (heatd2) and 90% ripe pod (heatd3) phases ranged from 819.7-933.7°C and 1,111.9-1,419.1°C (Table 4).

The data on the number of heat units was based on the age of the plants (Table 3); 2 local genotypes (Local Sumenep and Local Madura) required heat units to ripen pods 90% (heatd3) higher than other genotypes because having pods ripening 90% longer than other genotypes.

Local Gadis, Local Sumenep, and Local Madura are plant genotypes with a longer pod maturity than other genotypes. These genotypes required plant heat units to achieve 90% pod maturity at 1,235.42°C, 1,322.85°C, and 1,419.15°C.

Pod formation and yield potential of mung bean

The data on the fruit set and yield weight of mung bean plants were presented in Table 5. Several local genotypes, including Local Bima 2A and Local Madura, showed high flower formation. High flower formation in the generative phase does not guarantee high harvest weight because the fruitset of mung bean also determines the harvest weight. The number of flowers formed does not always correlate positively with each genotype's production yield. Plants that have many flowers, but if during the pod filling phase experience high flower loss or other factors that cause failure in the formation of pods and seeds, the production yield of that plant will also be low.

Table 4. The number of heat units (heat units °C) of mung bean plants needed until the first flowering phase (heatd1), first ripe pods (heatd2), and 90% ripe pods (heatd3)

Genotype	heatd1 (°C)	heatd2 (°C)	heatd3 (°C)
Vimal	544.6 ^a	853.2 ^a	1,134.25 ^a
Walet	544.6 ^a	869.7 ^a	1,144.78 ^a
Local Batang	544.6 ^a	842.0 ^a	1,122.98 ^a
Mentik Coklat	544.6 ^a	896.4 ^b	1,111.95 ^a
Local Bima 2A	599.6 ^a	901.7 ^b	1,117.88 ^a
Local Madura 0018	544.6 ^a	836.0 ^a	1,176.95 ^a
Local Pasar Kuningan	544.6 ^a	830.7 ^a	1,192.78 ^a
Local Majenang	550.4 ^a	858.5 ^a	1,138.45 ^a
Local Taragong	544.6 ^a	825.3 ^a	1,209.42 ^a
Local Gadis	654.1 ^b	923.0 ^b	1,235.42 ^{ab}
Arta koneng 01070	648.9 ^b	923.0 ^b	1,144.22 ^a
Pag Asa 2	544.6 ^a	819.7 ^a	1,127.92 ^a
Local Sampang	550.4 ^a	830.9 ^a	1,128.25 ^a
Local Sumenep	670.3 ^b	923.0 ^b	1,322.85 ^b
Local Madura	600.0 ^a	933.7 ^b	1,419.15 ^b
HSD5%	58.1	59.7	189.6

Note: Numbers in one column followed by the same letter are not significantly different from the Tukey test at the 5% level

Table 3. Variations in the time of appearance of the first flower, first ripe pod, and 90% ripe pod, as well as the degree of non-synchrony of pod ripening and the degree of indetermination of plant height of 15 local green bean genotypes

Genotype	Day After Planting (DAP) at:			Plant height (cm) at:			Degree of non-synchrony		Degree of indetermination for plant height (DDh)			
	First flower	First ripe pods	90% ripe pods	First flower	First ripe pods	90% ripe pods	Of pod maturity (DDd)		DDh1	DDh2	DDh3	DDh
	(D1)	(D2)	(D3)	(H1)	(H2)	(H3)	DDd1	DDd2				Average
Vimal	35.0 ^a	54.1 ^a	71.3 ^a	24.9 ^a	50.8 ^a	53.2 ^a	50.9 ^b	24.1 ^{ab}	50.8 ^a	53.2 ^a	4.5 ^a	36.18
Walet	35.0 ^a	55.0 ^a	72.0 ^a	24.5 ^a	54.8 ^a	59.6 ^a	51.3 ^b	23.5 ^{ab}	54.5 ^a	58.3 ^a	8.1 ^a	40.3
Local Batang	35.0 ^a	53.4 ^a	71.3 ^a	29.1 ^{ab}	60.4 ^{ab}	69.0 ^b	50.9 ^b	25 ^{ab}	50.7 ^a	57.2 ^a	12.2 ^a	40.03
Mentik Coklat	35.0 ^a	56.8 ^b	70.0 ^a	27.2 ^a	57.2 ^{ab}	64.3 ^b	50.0 ^{ab}	18.9 ^a	52.4 ^a	57.3 ^a	10.5 ^a	40.04
Local Bima 2A	38.3 ^a	56.9 ^b	71.7 ^a	34.1 ^b	62.0 ^{ab}	72.3 ^b	46.6 ^a	20.6 ^a	44.5 ^a	52.7 ^a	13.9 ^a	37.05
Local Madura 0018	35.0 ^a	53.0 ^a	74.0 ^a	26.5 ^a	49.3 ^a	55.0 ^a	52.7 ^b	28.3 ^{ab}	46.0 ^a	51.4 ^a	10.0 ^a	35.81
Local Pasar Kuningan	35.0 ^a	52.8 ^a	75.0 ^a	28.0 ^a	55.4 ^a	58.6 ^a	53.1 ^b	29.3 ^{ab}	49.5 ^a	52.3 ^a	5.4 ^a	35.74
Local Majenang	35.4 ^a	54.2 ^a	72.3 ^a	26.8 ^a	53.1 ^a	55.9 ^a	51.0 ^b	25.0 ^{ab}	49.4 ^a	52.0 ^a	5.0 ^a	35.46
Local Taragong	35.0 ^a	52.7 ^a	76.0 ^a	31.1 ^{ab}	57.6 ^{ab}	63.9 ^{ab}	53.3 ^b	29.7 ^b	46.1 ^a	51.1 ^a	9.2 ^a	35.45
Local Gadis	41.7 ^b	58.1 ^b	77.7 ^a	39.0 ^b	64.1 ^b	69.3 ^b	45.8 ^a	24.4 ^{ab}	38.9 ^a	43.5 ^a	7.6 ^a	29.98
Arta Koneng 01070	41.4 ^b	58.3 ^b	71.9 ^a	39.7 ^b	67.9 ^b	72.5 ^b	42.3 ^a	18.8 ^a	41.4 ^a	45 ^a	6.1 ^a	30.84
Pag ASA 2	35.0 ^a	52.0 ^a	71.7 ^a	33.8 ^{ab}	64.8 ^b	69.3 ^b	51.1 ^b	27.4 ^{ab}	47.6 ^a	51.1 ^a	6.5 ^a	35.05
Local Sampang	35.7 ^a	52.4 ^a	71.0 ^a	21.7 ^a	46.9 ^a	49.2 ^a	49.8 ^{ab}	26.1 ^{ab}	54.2 ^a	56.0 ^a	4.7 ^a	38.27
Local Sumenep	42.7 ^b	58.7 ^b	83.0 ^b	40.8 ^b	72.7 ^b	76.5 ^b	48.6 ^{ab}	29.3 ^{ab}	43.8 ^a	46.6 ^a	4.9 ^a	31.75
Local Madura	38.7 ^a	58.9 ^b	89.0 ^b	29.8 ^{ab}	59.6 ^{ab}	67.1 ^b	56.6 ^b	33.8 ^b	49.4 ^a	55.2 ^a	11.3 ^a	38.66
HSD5%	3.5	3.6	11.4	12.2	17	15	8.3	10.9	19	17.7	15.1	

Note: Numbers in one column followed by the same letter are not significantly different from the Tukey test at the 5% level. HSD: Honestly Significant Difference

Table 5. Information on fruit set and yield weight of mung bean plants based on the BNJ/Tukey test at 95% confidence interval

Genotype	First harvest flowers average	First harvest pod average	Second harvest flower average	Second harvest pod average	First harvest fruitset (%)	Second harvest fruitset (%)	Total fruitset (%)	Weight of 100 grains (g)	First harvest weight per plant (g)	Second harvest weight per plant (g)
Vima 1	39.78 ^{fgh}	18.33 ^f	31.44 ^h	10.89 ^g	46.46 ^{bc}	34.12 ⁱ	41.15 ^e	6.4 ^a	6.41 ^{ab}	3.80 ^{bc}
Walet	46.78 ^{ef}	19.78 ^f	67.11 ^{bc}	36.78 ^{cd}	43.00 ^{bcd}	53.83 ^{bcd}	49.40 ^{bcd}	5.72 ^b	5.98 ^{abc}	4.35 ^{bc}
Lokal Batang	40.78 ^{fg}	19.33 ^f	36.89 ^{gh}	16.22 ^{fg}	47.33 ^{bc}	44.46 ^{fgh}	46.10 ^{cde}	5.15 ^{cd}	4.61 ^{cde}	3.85 ^{bc}
Mentik Coklat	58.67 ^{cd}	20.89 ^{ef}	46.33 ^e	22.78 ^e	35.07 ^d	48.03 ^{defg}	41.21 ^e	3.77 ^e	3.70 ^{ef}	4.03 ^{bc}
Lokal Bima 2A	103.78 ^a	44.89 ^b	82.44 ^a	49.67 ^a	42.70 ^{bcd}	59.16 ^{bc}	50.00 ^{bc}	3.02 ^f	6.57 ^{ab}	6.95 ^{ab}
Lokal Madura 0018	52 ^{de}	24.22 ^{de}	69.78 ^b	43.56 ^b	46.25 ^{bc}	61.75 ^b	54.98 ^a	3.20 ^f	4.17 ^{de}	5.80 ^{ab}
Lokal Pasar Kuningan	31.89 ^h	12.89 ^g	40.33 ^{fg}	18.67 ^{ef}	40.49 ^{cd}	46.24 ^{efg}	43.87 ^{cde}	5.66 ^{bc}	3.75 ^{ef}	5.42 ^{abc}
Lokal Majenang	46.56 ^{ef}	18.67 ^f	43.55 ^{ef}	18.67 ^{ef}	40.47 ^{cd}	42.75 ^{ghi}	41.33 ^e	6.29 ^a	4.77 ^{cde}	4.26 ^{bc}
Lokal Taragong	40.33 ^{fgh}	19.11 ^f	35.22 ^{gh}	13.22 ^{fg}	47.53 ^{bc}	35.29 ^{hi}	41.61 ^{de}	5.96 ^{ab}	4.64 ^{cde}	3.25 ^c
Lokal Gadis	41.11 ^{fg}	21.11 ^{ef}	45.44 ^{ef}	18.44 ^{ef}	50.75 ^b	40.67 ^{ghi}	45.53 ^{cde}	5.15 ^{cd}	5.27 ^{bcd}	4.60 ^{abc}
Arta Koneng 01070	72.78 ^b	26.56 ^{cd}	62.11 ^{cd}	34.56 ^d	36.33 ^d	55.73 ^{bcd}	45.25 ^{cde}	4.91 ^d	6.99 ^a	4.93 ^{abc}
Pag Asa 2	39.44 ^{fgh}	14.33 ^g	36.78 ^{gh}	17.11 ^f	36.03 ^d	46.26 ^{efg}	41.31 ^e	5.90 ^{ab}	3.84 ^{de}	4.60 ^{abc}
Lokal Sampang	37.778 ^{gh}	12.89 ^g	37.22 ^{gh}	18.44 ^{ef}	34.43 ^d	49.87 ^{cdefg}	41.96 ^{cde}	4.06 ^e	2.50 ^f	3.57 ^{bc}
Lokal Sumenep	67.22 ^{bc}	28.33 ^c	72.78 ^b	39.11 ^{bcd}	42.62 ^{bcd}	57.56 ^{bcd}	48.99 ^{bcd}	3.07 ^f	4.18 ^{de}	5.77 ^{abc}
Lokal Madura	96.89 ^a	69.33 ^a	57.22 ^d	41.89 ^{bc}	71.43 ^a	73.13 ^a	72.16 ^a	4.67 ^d	5.78 ^{abc}	6.03 ^{ab}

Note: Numbers in one column followed by the same letter are not significantly different from the Tukey test at the 5% level

This study discovered that the genotypes with the most flowers in the first 2-3 weeks were Local Bima 2a (average 103,78 flowers) and Local Madura (average 96,89 flowers), but in the Local Bima 2A genotype, more than half of the flowers fell and did not reach the pod-filling stage. Genotypes that produce the maximum number of flowers within a certain period can give high yields based on the number of flowers and the number of pods formed. Some genotypes are also known to flower more only 2-3 weeks after the first flowering, and the number of flowers formed also varies between genotypes (Hwang et al. 2017). Therefore, knowledge of flowering patterns in mung bean plants is very important because it can assist in selecting genotypes with high yields. The potential yield of mung bean production is much higher than the amount obtained at harvest. Therefore, knowledge of flowering patterns in mung bean plants is very important because it can assist in selecting genotypes with high yields.

Several local genotypes, including the Local Bima 2A and Local Madura genotypes, showed high flower formation. High flower formation in the generative phase does not guarantee high harvest weight because harvest weight is also determined by the formation of pods (fruit set). Most genotypes (13) have fruit sets (<50%), and only two genotypes (Local Madura and Local Madura 0018) have fruit sets >50%. According to research (Mondal et al. 2011), around 55% to 85% of green bean flowers do not develop into ripe pods, which means that green bean plants generally have fruit sets below 45%. So, the two local genotypes above (Local Madura and Local Madura 0018) are plants with high fruitsets.

The seed weight factor also affects seed production yield per green bean plant. In the Bima 2A Local genotype, the dry weight of 100 grains was 3.02 g. In this study, the seed weight of the Local Bima 2A genotype was the smallest compared to the other genotypes. As for the Vimal variety, although the number of flowers was not as high as that of the Local Bima 2A and Local Madura, the first Vima 1 yield was higher than the Local Madura genotype; this happens because the seed weight of Vima 1 is quite large; namely, the weight of 100 seeds of Vimal green beans is 6.40 g. The relationship between flower intensity and flowering is very complex because it involves the number of flowers, the quality of the flowers, the number of flowers that appear, and competition for nutrients among the developing fruit.

Environmental factors such as wind and rainfall also affect fruitset in mung bean plants. Based on observational data from the Bogor climatology station, during the flowering and pod-filling phases in July 2022, maximum wind speed data was recorded on 16 July 2022, namely 6m/s in the moderate wind category (Hasse 2015), on 18 July 2022, in the fresh wind category with a speed of 8m/s and on 28 July 2022, it was 6m/s (data attached). Based on the Beaufort Scale, wind speeds above 6m/s are called moderate winds, which can shake tree branches (Hasse 2015; Mascitelli et al. 2019; Zhang et al. 2022). The Beaufort scale is an empirical calculation with a formula based on experiments that have been known since the

1920s when anemometers began to be used in general (Rodríguez et al. 2020).

Proximate analysis of mung beans

Proximate analysis obtained the highest protein content in genotype Local Taragong (23.06%) and the lowest in Local Madura (19.19%). The investigated protein contents were comparable to Guleria's 2017 research, every 100 g of green bean seeds contains 14.6-33.0 g of protein; vicilin and albumin are abundant in the protein component. Therefore, mungbean protein has the potential to be a unique, sustainable plant-based protein; however, further research is needed to fully understand its molecular and highly organized structure, as well as its functional characteristics, before any further advancement is made (Shrestha et al. 2023).

Based on the results of this research, ten local genotypes (Mentik Coklat, Local Bima 2A, Local Madura 0018, Local Pasar Kuningan, Local Majenang, Local Taragong, Local Gadis, and Arta koneng 01070) have a higher protein content compared to the developed variety (Vimal). Two local genotypes (Mentik Coklat and Local Taragong) have a higher protein content than the developed variety (Walet). This result is comparable with the research results from (Ebert et al. 2017), which stated that Mungbean landraces have higher protein, calcium, iron, zinc, carotenoid, and vitamin C levels at full maturity than developed varieties.

Seed moisture content varied from 6.98% to 11.87% among all genotypes. Local Majenang had the highest fat content (1.25%), while Local Madura had the lowest (0.05%). The starch content of mung beans is highest in Arta Koneng 01070 (4.43%) and lowest in Local Madura 0018 (3.88%). The complete results of the proximate analysis can be seen in Table 6.

Table 6. Proximate analysis results (protein, starch, fat, ash content, and seed moisture content)

Genotype	Protein	Starch	Ash content	Fat	Seed moisture
Vimal	20.55	4.27	3.93	0.90	9.11
Walet	22.01	4.07	4.06	0.75	8.22
Local Batang	20.39	4.21	4.10	0.90	8.15
Mentik Coklat	22.13	4.27	4.10	1.05	7.75
Local Bima 2A	20.87	4.04	4.02	0.81	8.63
Local Madura 0018	21.78	3.88	3.85	0.96	11.87
Local Pasar Kuningan	20.70	4.21	3.97	1.02	8.61
Local Majenang	20.86	4.24	4.01	1.25	8.64
Local Taragong	23.06	4.21	4.09	0.70	7.67
Local Gadis	21.18	3.95	4.30	0.69	9.20
Arta koneng 01070	21.63	4.43	4.33	0.64	6.98
Pag ASA 2	19.86	3.91	3.22	0.54	10.07
Local Sampang	20.56	4.08	3.41	0.95	8.33
Local Sumenep	20.60	4.01	3.41	0.93	9.02
Local Madura	19.19	3.95	2.73	0.05	9.56
Average	21.02	4.11	3.84	0.81	8.79
Min	19.19	3.88	2.73	0.05	6.98
Max	23.06	4.43	4.33	1.25	11.87

The visualization of the results is in Figure 3, a heatmap is an illustration of data whose values are represented by color. The concept is: red represents areas of high content, white represents transitions, and blue represents areas with lower or little content. From the proximate analysis heatmap, there are several plant groups. The top cluster, dominated by red, indicates high protein, starch, fat, and ash content. From the proximate analysis visualization, if we compare the upper and lower clusters, it can be seen that the water content is inversely proportional to the protein, starch, fat, and ash content. The bottom part is a cluster with a predominance of blue, which means a genotype group with low protein, starch, fat, and ash content. In this cluster, the exception is the water content, which is relatively high, as seen in the red visible blocks (Figure 3).

Analysis of genetic diversity with the help of molecular markers

Marker analysis

Polymorphic Information Content (PIC values) for evaluating microsatellite markers can be divided into three categories: highly informative if $PIC > 0.5$; moderately informative if $0.25 < PIC < 0.5$; and low informative if $PIC < 0.25$ (Savić et al. 2021; Zhang and Wang 2022; Oliya et al. 2023). PIC value is used to assess the informativeness of the marker developed; based on the PIC values obtained, there were eight SSR primers which highly informative for analyzing the genetic diversity of mung bean in this study, i.e. MBSSR136, MB SSR 021, DMB SSR 013, VrD1, MBSSR063, MB SSR 179, MBSSR 033 and Gaat 47 (Table 6). These markers showed high polymorphism and were very good at analyzing the diversity/relationships of mung beans in samples. Based on these results, it also has the potential to be used in studies of the genetic diversity of mung beans in general.

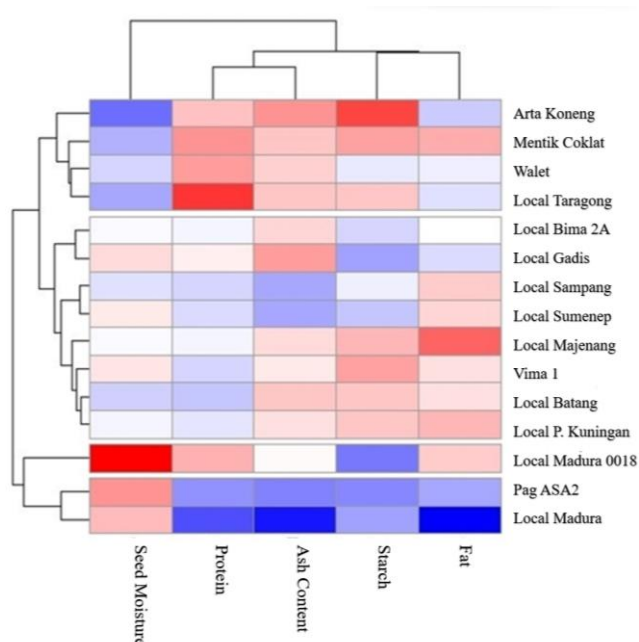


Figure 3. Protein, starch, fat, ash content, and seed moisture content of mung bean genotypes

Table 6. Heterozygosity (H) and Polymorphic Information Content (PIC)

Primer	H	PIC
MBSSR136	0.700	0.673
MB SSR 021	0.660	0.585
DMB SSR 013	0.766	0.734
VrD1	0.640	0.568
MBSSR063	0.746	0.708
MB SSR 179	0.791	0.758
MBSSR 203	0.500	0.449
MBSSR 033	0.843	0.824
MBSSR 015	0.165	0.152
GMES 1604	0.472	0.360
GMES 2225	0.507	0.415
Gaat 47	0.790	0.757

Based on genetic indices of SSR markers as shown in Figure 4, Ne values observed in the mungbean population ranged from 1.313-1.817 with an average value of 1.582. This value was categorized as high, reflecting that this mungbean population had a high diversity of alleles. This abundant allele indicated a major contribution of respective alleles in creating heterozygosity in this population. The Shannon index value (I) observed in the present study ranged from 0.310-0.637 (Fig.4) with an average value of 0.529 revealing that the heterozygosity of alleles in the population was moderately high. The value of Nei's diversity gene (h) ranged from 0.3240-0.4547 with an average of 0.3691 respectively.

Cluster analysis

Analysis of genetic diversity in mung bean plants can determine kinship relationships between genotypes. Kinship is a species' close relationship or pedigree of origin based on phenotypic and genotypic analysis. Based on the visualization results on the polyacrylamide gel scored as binary data, then analyzed using the NTSYS version 2.1 program, the genetic diversity of mung bean genotypes is shown in Figure 4.

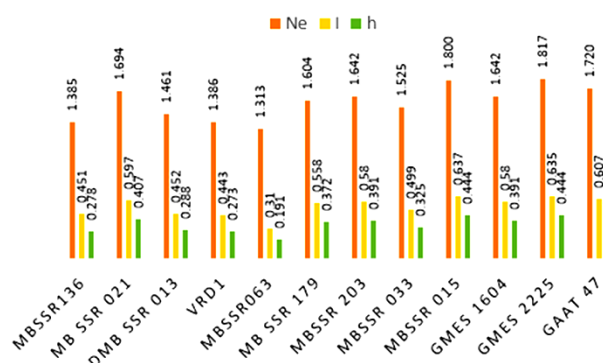


Figure 4. Ne (effective allele), h (heterozygosity), and I (Index Shannon) of SSR markers on mungbean

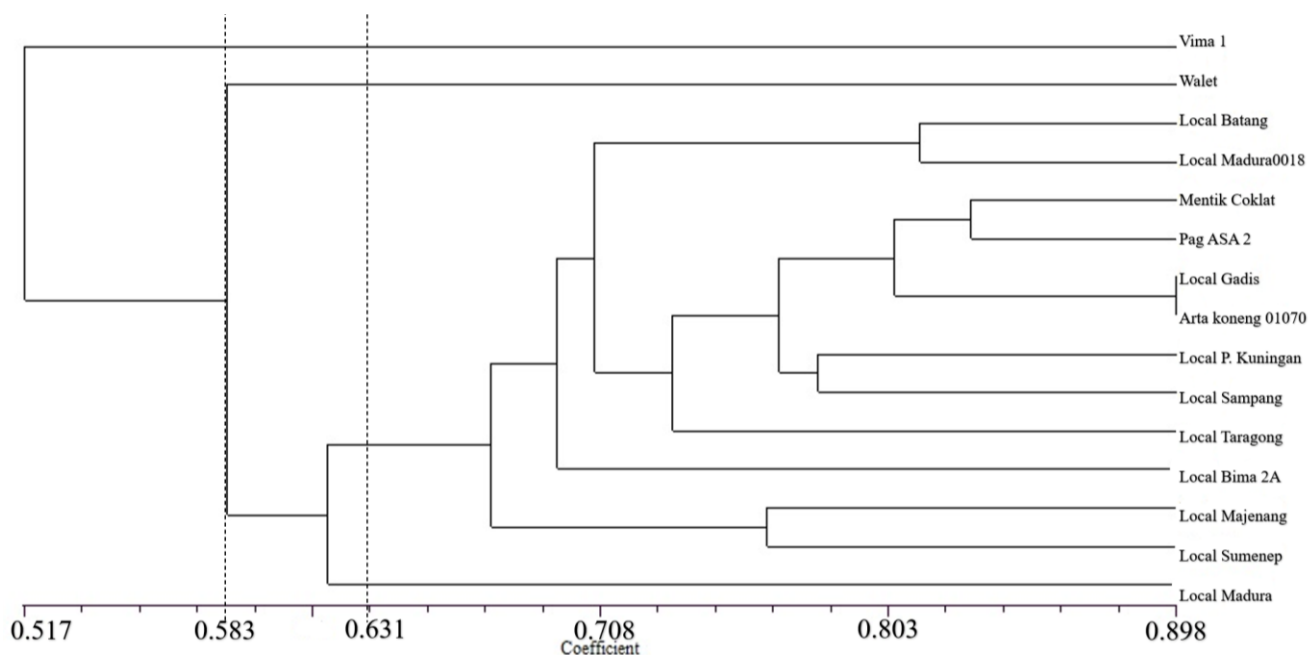


Figure 5. Dendrogram of the diversity of 15 mung bean genotypes based on SSR analysis

Based on the genetic similarity coefficient of 0.583, the superior varieties tested, namely Vima1 and Walet, were separated from other local genotypes. Vima1 and Walet have a distant degree of kinship with local mung beans. There are several clusters of mung beans with different levels of kinship; the higher the coefficient or genetic similarity value, the closer the genotypes.

Vima 1 is a crossbreed mung bean with introduced parents from Thailand, namely VC1973A as the male parent and VC2750A as the female parent (Balitkabi 2016; Reflinur et al. 2017). According to (Liu et al. 2022; Wang et al. 2022), VC1973A is a mung bean originating from Thailand and is often used as a cross-parent because this genotype has high yield potential, and its seeds are stored at the Asian Vegetable Research and Development Center (AVRDC). Mung bean seeds from AVRDC are widely spread to Southeast Asia, including Indonesia. One of the results of their crosses is the Vima1 variety, which is a superior soybean commodity released by Balitkabi Malang with the characteristics of early ripening simultaneously, resistant to powdery mildew, and tolerant of salinity (Trustinah et al. 2015; Balitkabi 2016).

Of all the plant samples observed, four plant groups/clusters were seen with a genetic diversity coefficient of 0.631. The first cluster is Vima 1, the second cluster is Walet, the third cluster is the Local Batang genotype, Local Madura 0018, Mentik Coklat, Pag ASA2, Local Gadis, Arta Koneng 01070, Local Pasar Kuningan, Local Sampang, Local Taragong, Local Bima 2A, Local Majenang, and Local Sumenep, as well as the fourth cluster, namely the Local Madura genotype. From phenotypic observations, the

Local Madura genotype had the highest level of total fruit set (72.16%) compared to the other 14 plants tested.

In conclusion, information on genetic diversity in several mung bean genotypes is important for basic research, conservation, utilization of germplasm resources, and crop improvement. The research results showed variations in the data on flowering pod maturity and plant height. The degree of non-synchrony of pod maturity from the first flowering to 90% of pod maturity (DDd1) indicates that the genotypes Local Bima 2A, Local Gadis, and Arta Koneng 01070 are more synchronized than the other genotypes. The heat units from planting to the first flower out (heatd1) ranged from 544.6-670.0°C. Mung bean cultivation from planting to the first ripe pod (heatd2) and 90% ripe pod (heatd3) phases ranged from 819.7-933.7°C and 1,111.9-1,419.1°C

There is also variation in protein, starch, and fat content in mung bean seeds, which indicates their potential as a rich source of plant breeding. Two local genotypes (Mentik Coklat and Local Taragong) have a higher protein content than the developed variety. The results of the phylogenetic tree/dendrogram of a diversity of 15 mung bean genotypes based on SSR analysis showed that the developed varieties used as comparison genotypes (Vima1 and Walet) appear to be separated from the other 13 local genotypes at a genetic similarity coefficient of 0.583. The informative SSR primers for analyzing the genetic diversity of mung beans in this study are MBSSR136, MB SSR 021, DMB SSR 013, VrD1, MBSSR063, MB SSR 179, MBSSR 033, and Gaat 47.

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