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The Morphological Characteristics of *Phaseolus lunatus* L. in Different Areas of East Java, Indonesia

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Abstract. *Phaseolus lunatus* L. is a type of legumes of which population is decreasing in Indonesia. The studies on analyzing *Phaseolus lunatus* L. from Indonesia are scarcely conducted. This current research aimed at revealing the diversity of *Phaseolus lunatus* L. which spreads across East Java, Indonesia. There are 15 accessions of *Phaseolus lunatus* L. which can be found in Madura, Probolinggo, Kediri, Tulungagung, and Malang. The morphological observation involved qualitative and quantitative parameters conducted in all collected accessions. The quantitative data were analyzed using one-way multivariate analysis of variance (MANOVA) by considering the accessions as independent variables and quantitative parameters as the dependent variables. The qualitative characters obtained were that all accessions had triangular shaped leaves and short green pods, whereas there were variations in the flower wings color (from white to purple), seed hull colors (from cream to black), and seed shape (kidney, cuboid, oval). Based on the results of the multivariate testing, the morphological characteristics of each *Phaseolus lunatus* L. accession were significantly different ($p < 0.005$). Based on the univariate testing results, there is a significant difference on the weight, length, width, and thickness of seed, the length, and width of the leaf, and also the length and width of the pod ($p < 0.005$). The findings have shown that there are differences in various morphological parameters which can be used as the foundation for further researches.

Keywords: *Phaseolus lunatus* L., morphological observation, qualitative, quantitative parameters, east java

1. Introduction

Indonesia is a country with extensively diverse local food source [1,2]. Surprisingly, Indonesian's protein sufficiency is considered low [3–6]. This contradictive condition is stimulated by several factors, including the fact that Indonesia's population is considerably high and that protein from animals are less affordable [7,8], and also the lack of alternatives for local plant-based protein sources [9–11]. Apart from the exposed problem, Indonesia is a country which is rich in legume varieties [1,12]. Hence, continuous exploration of the potential of legumes in Indonesia is a solution to overcome the problem of protein insufficiency in this country.

Phaseolus lunatus L. (also known as *koro* beans, lima beans, or butter beans) is a type of legume which is potential to become the food source for Indonesians as it is rich in nutrition [7,13–17]. This plant contains an extra floral nutrition in its leaf stipule [18,19] and is completed with several



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important nutrition contents, such as fiber [20–24] and protein [22,25–27]. It is also rich in anti-oxidant [21,28] and other substances which can help prevent illnesses [26,28–30]. These nutrition and substances make *P. lunatus* L. a popular source of protein in many countries, such as in Africa and Latin America [31,32].

Unfortunately, its potential as a food source rich in nutrition is under-appreciated by most Indonesians [33]. This plant is only cultivated for seeding and is hardly used as a food source because of its anti-nutrition contents [34–36]. In fact, with proper processing, the anti-nutrition can be managed [36–38]. The fact that *P. lunatus* is a promising alternative source of protein is contradictory to the fact that the plant is decreasing in population in Indonesia. Apart from being less known, government's policy in the cultivation of grains is mostly focused on peanuts (*Arachis hiphogea*), soybeans (*Glicine maks* L.) and mung beans (*Phaseolus radiatus*) [39] which results in farmers growing other types of crops. This way, local grain products are less appreciated in cultivation, production, and conservation.

In addition to the decreasing number of *P. lunatus* L. population, studies concerning the diversity and diversification of beans in Indonesia are limited in all sorts. In other countries, research programs are conducted extensively to study this species. Related to the diversity, some research on *P. lunatus* L. have been conducted in North America [40,41], Central America [42], Latin America [40,41], Caribbean countries [40], Europe [43], and Africa [40]. In Indonesia, most researches on *P. lunatus* L. are still limited to the substances contained in the plant [7,9,15,27,37], and it is potential as an alternative source of food [16,17].

Concerning the above-elaborated background, a continuous effort in conserving *P. lunatus* L. To maintain biodiversity in Indonesia is of much required. This effort helps improve people's knowledge of food sources and alternative food sources which are grain-based. Therefore, it is of urgency to conduct research which starts from a survey of diversification of *P. lunatus* L. in all areas of Indonesia to accurately diagnose its population and the varieties as the initial step of conservation. Furthermore, species diversity study is an interesting yet important field of research [42,44,45]. Diversity and the relation among *Phaseolus* species in Indonesia are hardly understood. Accordingly, this current research aims at investigating the morphological diversity of *P. lunatus* L. in East Java, Indonesia.

2. Methods

This research was initiated by conducting a descriptive observation through a survey. *P. lunatus* L. was chosen as the object of the investigation, collected from some areas around East Java, namely Tulungagung, Kediri, Malang, Probolinggo, and Madura. From each population, the sample was taken in order to observe the morphological characteristics of the plant meticulously. The morphological analysis involved several observations on both quantitative and qualitative characteristics of the plant. Morphological characteristics were identified by using "Handbook on Evaluation of Germplasm" guide book, subchapter "Characterization of *Phaseolus* Accessions" (page 29-43) [46], which include: 1) Plant types, 2) leaf form, 3) flower, 4) pods, and 5) seeds. Morphological analysis was conducted in Biology Laboratory, University of Muhammadiyah Malang. The data about qualitative characteristics contained information about the types of plant, leaf form, leaf wing color, pod color, seed hull color, and seed shape. The data about quantitative characteristics, on the other hand, contained the information about the seed length, seed width, seed dimension, seed weight, leaf length, leaf width, pod length, and pod width.

The diversity of quantitative parameters was analyzed to detect the differences in morphological characteristics in the collected accessions. The data resulted from the quantitative analysis were then analyzed by using one-way multivariate analysis of variance (one-way MANOVA) with the significance level (α) 0.05. The accessions of *P. lunatus* L. were used as the independent variables; while the quantitative parameters obtained were used as the dependent variables. If the analysis results show a significant difference, the analysis was proceeded to the least significant difference (LSD) as a posttest. The data analysis was assisted by SPSS 22.0 for windows.

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3. Results and Discussion

The results of the survey in East Java inform that some areas which still cultivate *P. lunatus* L are Madura and Probolinggo; while Tulungagung, Kediri, and Malang do not cultivate this plant as much. From the survey, 15 accessions of *P. lunatus* L. from some areas in East Java were obtained. In Table 1, the morphological characteristics of the qualitative parameters of the 15 accessions are presented. Based on the table, it can be seen that generally, the leaf of *P. lunatus* L. which grows in East Java is triangular in shape, 100% ravine type, 70% kidney-shaped seed, 30% oval-shaped, 6% cream-colored seed, 50% dark brown color, 20% blackish, 6% maroon, 6% purplish, and 12% with spots and stripes, 100% young pod in green color, 100% short pod tip, 40% flower wing in white color, 30% violet, and 30% yellowish. The diversity of morphological characteristics observed from the accessions in this current research has confirmed the description of *P. lunatus* L. characteristics in the references discussing the types of agricultural and medicinal plants [47–50].











Table 1. The qualitative characteristics of *P. lunatus* L. in East Java






Code	Plant Types	Leaf Shapes	Flower Wings	Pod Colors (Young)	Pod Tip Shapes	Seed Hull Colors	Seed Shapes	Cultivation Status
2	Vine	Triangular	White	Green	Short	Black	Kidney	Rare
4	Vine	Triangular	Yellow	Green	Short	Brown-Maroon	Flat Kidney	Rare
7	Vine	Triangular	White-Yellow	Green	Short	Brown	Flat Kidney	Rare
8	Vine	Triangular	White-Purple	Green	Short	Maroon	Curvy Kidney	Rare
12	Vine	Triangular	White-Purple	Green	Short	Maroon, spots	Oval-ellipse	Rare
13	Vine	Triangular	White-Purple	Green	Short	Dark brown	Oval medium	Rare
14	Vine	Triangular	White-Purple	Green	Short	Brown, stripes	Oval medium	Rare
16	Vine	Triangular	Purple	Green	Short	Purple-Black	Wide Flat Kidney	Rare
18	Vine	Triangular	Purple	Green	Short	Dark purple	Flat Kidney	Rare
19	Vine	Triangular	White	Green	Short	Cream	Medium Flat Kidney	Common
Prb.1	Vine	Triangular	White	Green	Short	½ white, ½ black	Flat Cuboid	Rare
Prb.2	Vine	Triangular	Purple	Green	Short	Dark purple	Flat Kidney	Rare
Prb.3	Vine	Triangular	Yellow	Green	Short	Dark brown	Wide Flat Kidney	Medium
Prb.4	Vine	Triangular	White-Purple	Green	Short	Light brown, spots, black tints	Oval	Medium
Prb.5	Vine	Triangular	Yellow	Green	Short	Cream, stripes	Wide Flat Kidney	Medium

Besides the qualitative parameters, the quantitative parameters were also observed. The data from quantitative observation are presented in Table 2. Of the 15 accessions collected, the plants can be categorized into culti-groups with the following characteristics: 1) Potato, in which the seeds are small and round (35.5 g/100 seed weight, 9 mm in length, 8 mm in width); 2) Potato-Sieva, of which seeds are small-ovalish 36.3 g/100 seed weight, 11 mm in length, 8 mm in length); 3) Sieva, of which seeds are oval-kidney-shaped and medium-sized (30-45.3 g/100 seed weight, 12 mm in length, 9-10 mm in

width); 4) Sieva-Big Lima, oval-kidney-shaped and medium-wide-sized, (77.5 g/100 seed weight, 17 mm in length, 11 mm in width); and 5) Big Lima, the size and shape of the seeds are relatively big (100-110 g/100 seed weight, 25 mm in length, 14 mm in width).

Table 2. The morphological characteristics of seed, leaf, and pod of *P. lunatus* L. in East Java and its cultivation status

Code	Gene pool	Image	Seed Length (cm)	Seed Width (cm)	Seed Dimension (cm)	Seed Weight gr/seed	Leaf Length (cm)	Leaf Width (cm)	Pod Length (cm)	Pod Width (cm)
2	Sieva-big		1.57	1.02	0.26	0.49	7.8	6.9	9.2	3.3
4	Sieva-big		2.38	1.51	0.31	0.41	9.0	6.0	13.4	5.4
7	Sieva-big		1.62	1.13	0.19	1.49	8.25	5.25	10.1	3.1
8	Sieva-Big		1.56	1.20	0.52	0.55	6.75	5.1	9.2	3.1
12	Potato-Sieva		1.03	0.98	0.31	0.35	8.1	2.5	8.1	2.6
13	Potato-Sieva		1.00	0.92	0.40	0.35	7.8	2.25	8.1	2.5
14	Potato-Sieva		1.16	1.00	0.21	0.43	9.9	1.4	8.3	2.8
16	Sieva-big		1.65	1.39	0.35	0.7	12.5	7.9	16.3	6.7
18	Sieva-Big		1.5	1.18	0.47	0.47	7.2	5.25	13.6	3.8
19	Sieva-big		1.57	1.08	0.23	0.46	8.1	6.0	15.08	4.1

Code	Gene pool	Image	Seed Length (cm)	Seed Width (cm)	Seed Dimension (cm)	Seed Weight gr/seed	Leaf Length (cm)	Leaf Width (cm)	Pod Length (cm)	Pod Width (cm)
Prb 1	Sieva-Big		1.51	1.41	0.36	0.76	9.2	7.6	13.7	3.8
Prb 2	Sieva-Big		1.50	1.00	6.00	0.66	12	8.2	8.8	2.8
Prb 3	Big lima		2.20	1.40	0.43	1.05	12.8	8.1	15.9	5.8
Prb 4	Sieva		1.20	1.00	0.43	0.42	14.6	3.4	9.1	3.2
Prb 5	Big lima		2.50	1.50	0.45	1.79	11.2	9.2	16.2	6.2

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The quantitative data were analyzed using one-way MANOVA. The results of the multivariate and univariate testing are presented in Table 3. It is noticeable that the results of the multivariate testing show that the morphological characteristics of *P. lunatus* L. accessions taken from some areas in East Java possess significant difference [$F(112, 909) = 734.795, p < 0.005; \eta^2 = 0.981$]. Furthermore, based on the univariate testing results presented in Table 3, it can be seen that the morphological parameters showing significant difference among the accessions of *P. lunatus* L. are the weight of seed [$F(14, 135) = 777.910, p < 0.005; \eta^2 = 0.988$], seed length [$F(14, 135) = 92.883, p < 0.005; \eta^2 = 0.906$], seed width [$F(14, 135) = 26.139, p < 0.005; \eta^2 = 0.731$], seed dimension [$F(14, 135) = 32.124, p < 0.005; \eta^2 = 0.769$], leaf length [$F(14, 135) = 407.879, p < 0.005; \eta^2 = 0.977$], leaf width [$F(14, 135) = 459.574, p < 0.005; \eta^2 = 0.979$], pod length [$F(14, 135) = 460121.214, p < 0.005; \eta^2 = 0.999$], and pod width [$F(14, 135) = 81695.430, p < 0.005; \eta^2 = 0.999$]. This has indicated the differences in the pod, seed, and leaf. Moreover, it has also been indicated that those parameters can be used to differentiate the genotypes of *P. lunatus* L. growing in East Java. The summary of post test result using LSD testing is presented in Table 4.

Based on Table 4, the information about the diversity of morphological characteristics of *P. lunatus* L. accessions is obtained. First, some accessions possess characteristics which are not significantly different on the observed parameters. Secondly, an accession which is the biggest in one parameter is not necessarily the biggest in other parameters. Thirdly, when comparing the seed and leaf, the most visible and significant difference of accessions is on the pod. The diversity of *P. lunatus* L. found in this research is in line with some previous studies in several which reports that there is a morphological diversity of *P. lunatus* L. from various regions, both in Indonesia [51] and outside Indonesia [52]. This information is

reinforced by the genetic diversity of *P. lunatus* L. which is also revealed in various other studies [53,54].

Table 3. The results of one-way MANOVA testing on the quantitative data of morphological characteristics of *P. lunatus* L. in East Java

Tests	Hypothesis df	Error df	F	Sig.	η^2
Multivariate	112	909	734.795	< 0.005	0.981
Seed weight	14	135	777.910	< 0.005	0.988
Seed length	14	135	92.883	< 0.005	0.906
Seed width	14	135	26.139	< 0.005	0.731
Seed dimension	14	135	32.124	< 0.005	0.769
Univariate					
Leaf length	14	135	407.879	< 0.005	0.977
Leaf width	14	135	459.574	< 0.005	0.979
Pod length	14	135	460121.214	< 0.005	0.999
Pod width	14	135	81695.430	< 0.005	0.999

Table 4. The summary of LSD testing on the quantitative parameters of *P. lunatus* L accessions in East Java

Accessions	Seed Weight	Seed Length	Seed Width	Seed Dimension	Leaf Length	Leaf Width	Pod Length	Pod Width
2	0.491 ^a	1.570 ^d	1.020 ^{abc}	0.259 ^{bc}	7.820 ^c	6.920 ^f	9.209 ^e	3.307 ^f
4	0.412 ^c	2.380 ^g	1.510 ^g	0.311 ^{cd}	9.009 ^e	6.015 ^e	13.399 ^g	5.397 ⁱ
7	1.489 ⁱ	1.620 ^{de}	1.130 ^{cde}	0.190 ^a	8.265 ^d	5.255 ^d	10.113 ^f	3.116 ^d
8	0.572 ^e	1.568 ^d	1.210 ^e	0.471 ^g	6.760 ^a	5.090 ^d	9.210 ^e	3.109 ^d
12	0.350 ^b	1.030 ^{ab}	0.980 ^{ab}	0.309 ^{cd}	8.090 ^{cd}	2.500 ^b	8.109 ^a	2.613 ^b
13	0.350 ^b	1.010 ^a	0.920 ^a	0.399 ^{ef}	7.800 ^c	2.250 ^b	8.101 ^a	2.495 ^a
14	0.430 ^{cd}	1.160 ^{bc}	1.000 ^{ab}	0.205 ^{ab}	9.900 ^f	1.400 ^a	8.306 ^b	2.809 ^c
16	0.700 ^f	1.750 ^e	1.390 ^f	0.351 ^{de}	12.500 ⁱ	7.900 ^{gh}	16.306 ^m	6.709 ^l
18	0.4660 ^d	1.500 ^d	1.190 ^{de}	0.471 ^g	7.200 ^b	5.250 ^d	13.611 ^h	3.810 ^g
19	0.460 ^d	1.580 ^d	1.090 ^{bcd}	0.212 ^{ab}	8.100 ^{cd}	6.000 ^e	15.074 ^j	4.110 ^h
Prb 1	0.760 ^g	1.530 ^d	1.410 ^{fg}	0.420 ^{fg}	9.150 ^e	7.600 ^g	13.700 ⁱ	3.800 ^g
Prb 2	0.660 ^f	1.510 ^d	1.020 ^{abc}	0.600 ^h	12.170 ^h	8.200 ^h	8.809 ^c	2.811 ^c
Prb 3	1.050 ^h	2.200 ^f	1.400 ^{fg}	0.440 ^{fg}	12.800 ⁱ	8.140 ^h	15.909 ^k	5.807 ^j
Prb 4	0.432 ^{cd}	1.180 ^c	1.010 ^{ab}	0.470 ^g	14.600 ^j	3.400 ^c	9.107 ^d	3.205 ^c
Prb 5	1.800 ^j	2.490 ^g	1.480 ^{fg}	0.460 ^g	11.140 ^g	9.200 ⁱ	16.208 ^l	6.208 ^k

Note: Scores followed by the same alphabets show that there is no significant difference on $p < .05$

The morphological differences of accessions can be caused by two factors, namely the genetic composition and the condition of the environment. Every plant accession generally carries different allele variations. The different types of allele are sometimes characterized as different phenotypic [55–57]. Environment condition which includes the differences of chemical compositions available in the soil and other physical parameter differences present on the environment will also interact with the genetic factor to characterize the living beings, including plants [58]. The average of temperature, moisture, light intensity, soil pH, soil nutrition, and the other physical parameter from one location to another can be varied. Concerning variations in physical parameters, those various external factors fluctuate strongly and have an impact on the diversity of plant morphological trait [58,59]. Furthermore, it was explained, variations in environmental conditions can affect plant characteristics, both directly and indirectly. Direct influence occurs when the environment influences the primary

development process, while indirect effects occur when the environment directs developmental adaptation.[59,60].

Based on the findings of this current research, it has been noticeable that *P. lunatus* L. in East Java possesses high diversity. The diversity needs to be maintained as a part of the biodiversity assets of Indonesia. The findings show that there are differences in *P. lunatus* L. morphological parameters which can be used to design further researches on the diversity or the phylogenetic relations of legume accessions in Indonesia. Moreover, the morphological characteristic differences in this research need to be confirmed with genetic diversity study, recommended for further researches. It is necessary to further verify the correlation between morphological diversity and the genetics of *P. lunatus* L.

4. Conclusion

In this research, the diversity of *P. lunatus* L. accessions has been analyzed. From the survey, 15 accessions of *P. lunatus* L. were obtained from some areas in East Java, namely Malang, Probolinggo, Kediri, Tulungagung, and Madura. Regarding qualitative parameters, all *P. lunatus* L. accessions are vine plant with triangular leaf shape and possess young pods in green color with short tip. Besides, majority of the plants have flower wings in darker shades with the seed shape similar to that of kidney. Regarding quantitative parameters, the morphological characteristics of the accessions have shown significant value [$F(112, 909) = 734.795, p < 0.005; \eta^2 = 0.981$]. From the univariate testing results, there are differences in the pod, seed, and leaf width.

Further researches investigating the diversity of *P. lunatus* L. from other parameters are highly recommended. The parameters could be protein and genetic diversities. Such research will illustrate the diversity of *P. lunatus* L. in Indonesia. Furthermore, it is recommended that further researches aiming at analyzing the phylogenetic relation among the accessions of *P. lunatus* L. which grow all around Indonesia be conducted.

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