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
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
substrates 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 contradictive
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 hypogea),
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
counter the discussion 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.
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          | $\frac{1}{2}$ white, $\frac{1}{2}$ 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           |
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 weight \[ F (14, 135) = 26.139, p < 0.005; \eta^2 = 0.731 \], 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

| 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           |
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. | ηp² |
|------------------------|---------------|----------|--------|------|-----|
| 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 |
|------------|-------------|-------------|------------|----------------|-------------|------------|------------|-----------|
| Prb 1      | 0.491b      | 1.570d      | 1.020ab    | 0.250bc        | 7.820c      | 6.920f     | 9.200e     | 3.307f    |
| Prb 2      | 0.412c      | 2.380f      | 1.510d     | 0.311cd        | 9.009e      | 6.015e     | 13.399d    | 5.397f    |
| Prb 3      | 1.489r      | 1.620qe     | 1.130de    | 0.190a        | 8.265d      | 5.255d     | 10.113c    | 3.116d    |
| Prb 4      | 0.572e      | 1.568d      | 1.210e     | 0.471f        | 6.760a      | 5.090d     | 9.210e     | 3.109d    |
| Prb 5      | 0.350b      | 1.030ab     | 0.980b     | 0.309cd       | 8.090d      | 2.500b     | 8.109b     | 2.613b    |
| Prb 6      | 0.350b      | 1.010a      | 0.920a     | 0.399c        | 7.800c      | 2.250b     | 8.101b     | 2.495b    |
| Prb 7      | 0.430cd     | 1.160bc     | 1.000b     | 0.205ab       | 9.900f      | 1.400c     | 8.306b     | 2.809f    |
| Prb 8      | 0.700f      | 1.750f      | 1.390f     | 0.351de       | 12.500h     | 7.900b     | 16.306h    | 6.709d    |
| Prb 9      | 0.4660d     | 1.500d      | 1.190e     | 0.471f        | 7.200b      | 5.250d     | 13.611b    | 3.810e    |
| Prb 10     | 0.460d      | 1.580d      | 1.090bcd   | 0.212ab       | 8.100d      | 6.000c     | 15.074d    | 4.110b    |
| Prb 11     | 0.760f      | 1.530d      | 1.410g     | 0.420g        | 9.150e      | 7.600f     | 13.700h    | 3.800e    |
| Prb 12     | 0.660f      | 1.510d      | 1.020bc    | 0.600b        | 12.170h     | 8.200b     | 8.809b     | 2.811e    |
| Prb 13     | 1.050b      | 2.200f      | 1.400f     | 0.440g        | 12.800h     | 8.140c     | 15.909h    | 5.807j    |
| Prb 14     | 0.432cd     | 1.180f      | 1.010ab    | 0.470f        | 14.600d     | 3.400e     | 9.107d     | 3.205s    |
| Prb 15     | 1.800b      | 2.490e      | 1.480g     | 0.460e        | 11.140e     | 9.200f     | 16.208g    | 6.208k    |

*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 \( P. \text{lunatus} \) \( L. \) 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 \( P. \text{lunatus} \) \( L. \) 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 \( P. \text{lunatus} \) \( L. \).

4. Conclusion
In this research, the diversity of \( P. \text{lunatus} \) \( L. \) accessions has been analyzed. From the survey, 15 accessions of \( P. \text{lunatus} \) \( L. \) were obtained from some areas in East Java, namely Malang, Probolinggo, Kediri, Tulungagung, and Madura. Regarding qualitative parameters, all \( P. \text{lunatus} \) \( L. \) 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 \( P. \text{lunatus} \) \( L. \) from other parameters are highly recommended. The parameters could be protein and genetic diversities. Such research will illustrate the diversity of \( P. \text{lunatus} \) \( L. \) in Indonesia. Furthermore, it is recommended that further researches aiming at analyzing the phylogenetic relation among the accessions of \( P. \text{lunatus} \) \( L. \) which grow all around Indonesia be conducted.

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